

Supplementary Materials for

**A VASP-Rac-Soluble Guanylyl Cyclase Pathway Controls cGMP
Production in Adipocytes**

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The PDF file includes:

- Fig. S1. Role of VASP in brown adipocyte differentiation.
- Fig. S2. *sGCβ1* promoter analysis.
- Fig. S3. White adipogenic differentiation of MEFs and white preadipocytes.
- Fig. S4. Effect of VASP ablation on ERK1/2 signaling.
- Fig. S5. Expression of *PGC-1α* and abundance of β_3 -AR in BAT.

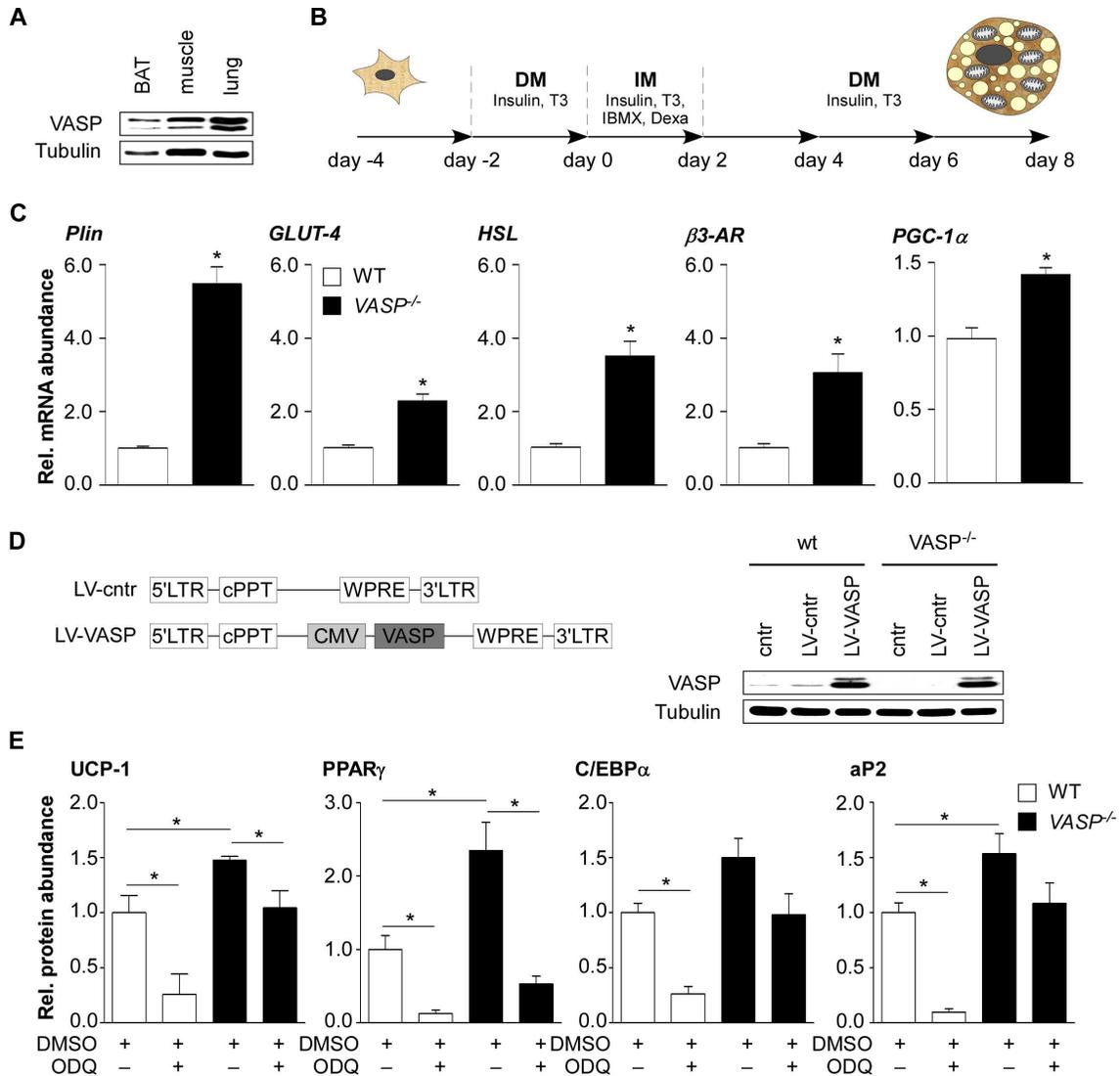


Figure S1. Role of VASP in brown adipocyte differentiation. (A) Western blot analysis of VASP abundance in BAT, skeletal muscle and lung from newborn WT mice. (B) Brown adipocyte differentiation (day -4 to day 8) and the media used (DM, differentiation medium; IM, induction medium; IBMX, isobutylmethylxanthine; Dexa, dexamethasone; T3, triiodothyronine). (C) RQ-PCR analysis of the mRNA abundance of *perilipin* (*Plin*), *glucose transporter type 4* (*GLUT-4*), *hormone-sensitive lipase* (*HSL*) and β -adrenergic receptor (β -AR) as well as of the mitochondrial marker *PGC-1 α* in differentiated WT and *VASP*^{-/-} brown adipocytes. Data are represented as means \pm SEM; * $p < 0.05$; $n=3$ sets of adipocytes.

(D) Lentiviruses used for restoration of VASP (left). The control vector does not contain a promoter or transgene (LV-cntr). LV-VASP expresses full-length VASP under control of the cytomegalovirus (CMV) promoter. LTR, long terminal repeat; cPPT, central polypurine tract; WPRE, post-transcriptional regulatory element of the woodchuck hepatitis virus. VASP protein abundance in non-infected (control, cntr), LV-cntr-, and LV-VASP-infected WT, and *VASP*^{-/-} brown preadipocytes (day 0) (right). (E) Densitometric analysis of marker protein UCP-1, PPAR γ , C/EBP α , and aP2 abundance in differentiated brown WT and *VASP*^{-/-} adipocytes treated with the sGC inhibitor ODQ (see Western blot analysis in Figure 4A). Data were normalized to tubulin and are represented as means \pm SEM; * $p < 0.05$; n=4 sets of adipocytes.

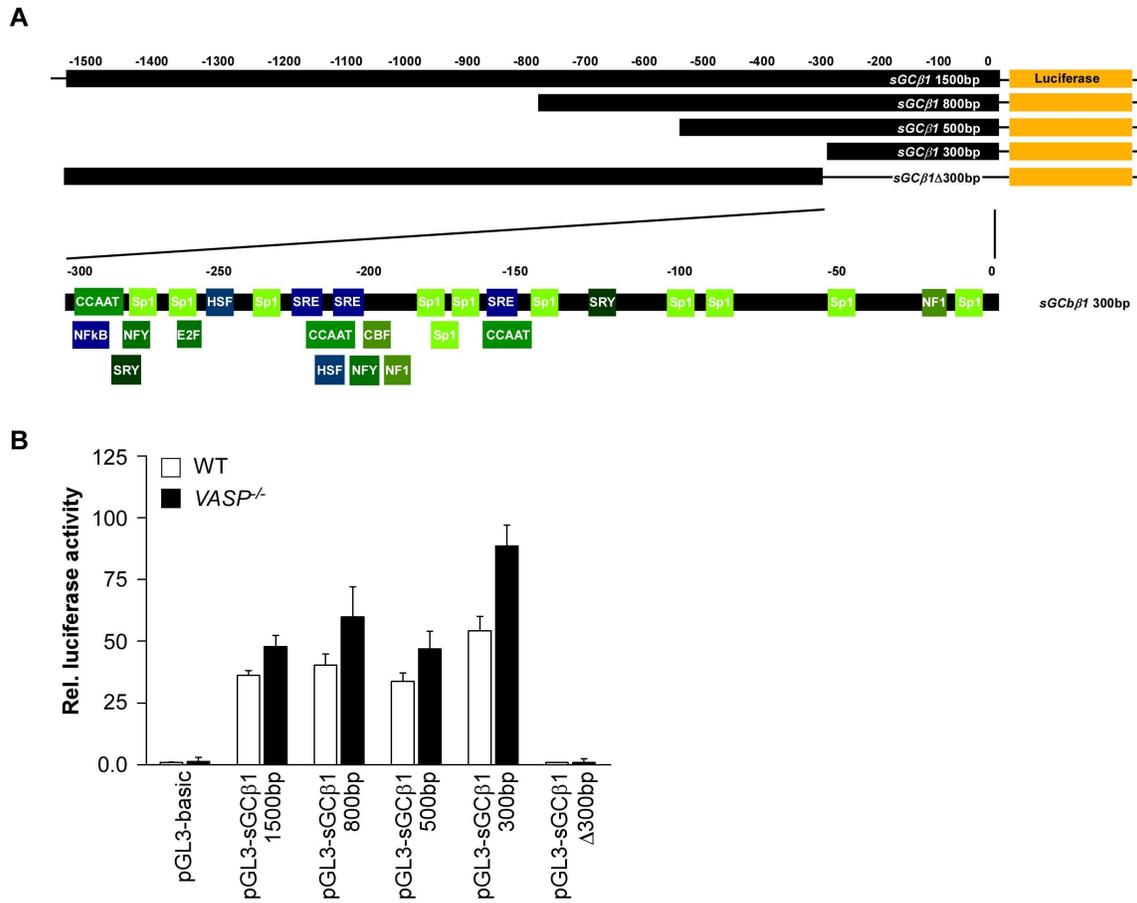


Figure S2. *sGCβ1* promoter analysis. (A) Overview on generated *sGCβ1* promoter deletion variants and putative transcription factor binding sites located in the proximal 300 bp promoter fragment. (B) Transcriptional activity of the *sGCβ1* promoter variants was determined by dual luciferase reporter assays. Data were normalized to pGL3-basic reporter activity and are represented as means \pm SEM. n=3 independent experiments.

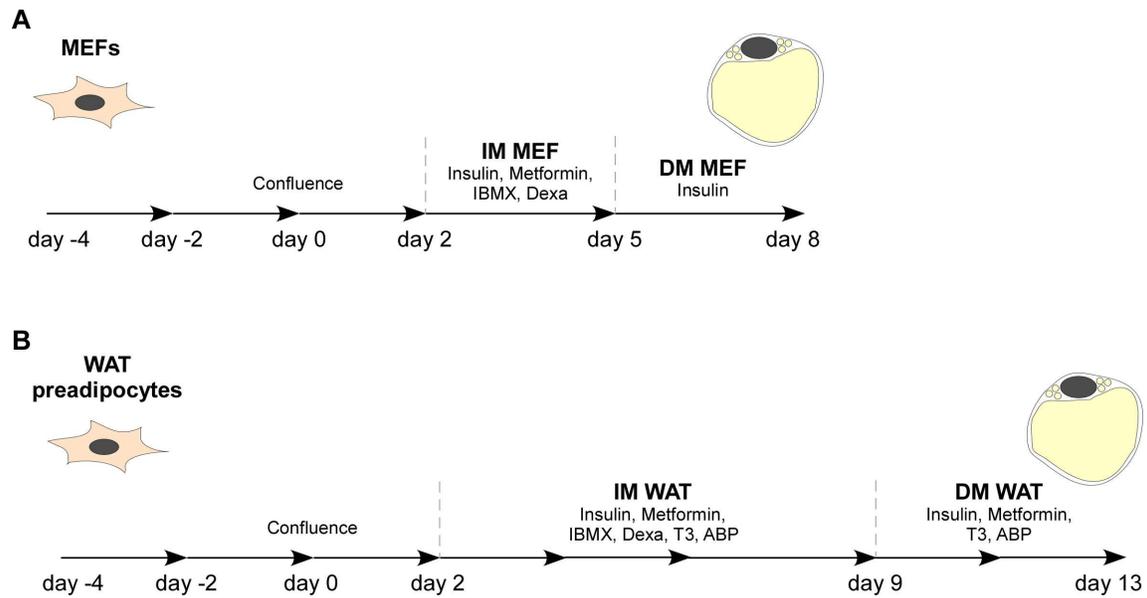


Figure S3. White adipogenic differentiation of MEFs and white preadipocytes. (A) Adipogenic differentiation of MEFs (day -4 to day 8) and the media used (DM MEF, MEF differentiation medium; IM MEF, MEF induction medium; IBMX, isobutylmethyl-xanthine; Dexa, dexamethasone). (B) Adipogenic differentiation of WAT preadipocytes (day -4 to day 13) and the media used (DM WAT, WAT preadipocyte differentiation medium; IM WAT, WAT preadipocyte induction medium; IBMX, isobutylmethyl-xanthine; Dexa, dexamethasone; T3, triiodothyronine; ABP, L-ascorbate, D-biotin, panthothenate).

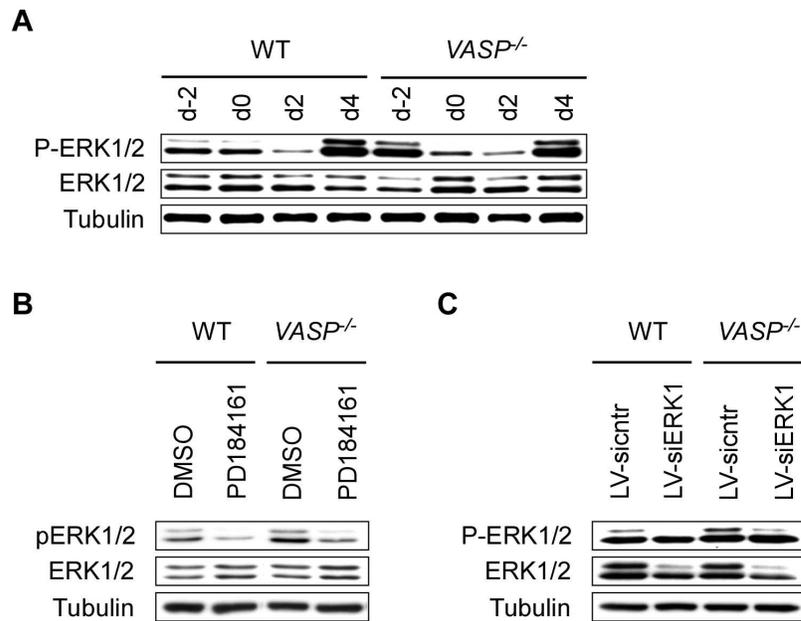


Figure S4. Effect of VASP ablation on ERK1/2 signaling. (A) Western blot analysis of phosphorylated ERK1/2 (P-ERK1/2) in WT and *VASP*^{-/-} cells at different stages of differentiation (day -2 to day 4). n=3 independent sets of cells. (B) Western blot analysis of ERK1/2 activity in WT and *VASP*^{-/-} preadipocytes incubated with 5 μ M PD184161 or vehicle for 24 hours using a phosphorylation-specific antibody against ERK1/2. (n=3 independent sets of cells) (C) Western blot analysis of ERK1/2 activity and abundance in WT and *VASP*^{-/-} preadipocytes 24 hours after transduction with a lentivirus expressing an siRNA against ERK1 (LV-siERK1) or a control siRNA (scrambled; LV-sicnr), respectively. n=3 independent sets of cells.

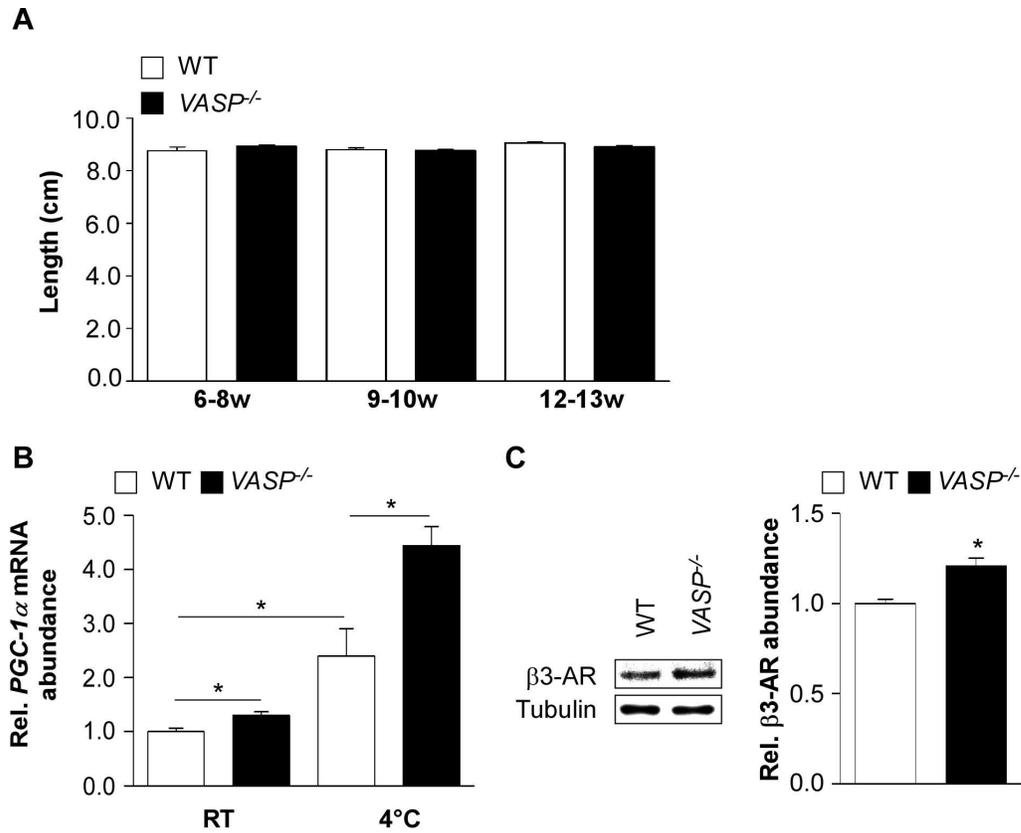


Figure S5. Expression of *PGC-1α* and abundance of β_3 -AR in BAT. (A) Body length of male wild-type (WT, open bars) and *VASP*^{-/-} (black bars) mice, of different age (6-8-, 9-10- or 12-13-week-old), was measured from the nose to the anus; bars represent the mean value \pm SEM (n=3-13 mice). (B) Abundance of *PGC-1α* mRNA in BAT of WT and *VASP*^{-/-} mice exposed to RT and 4°C as analyzed by RQ-PCR. (C) Western blot analysis of β_3 -AR abundance in BAT from WT and *VASP*^{-/-} mice.