**SUPPLEMENTARY INFORMATION**

# Transgenic overexpression of VEGF-C induces weight gain and insulin resistance in mice

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## SUPPLEMENTARY METHODS

### Whole mount immunofluorescence stains

Briefly, 1 cm long pieces of small intestine were dissected and cut longitudinally, washed with PBS and fixed in 4% PFA for 1 h at 4ºC. The samples were then blocked with a mixture of 5% donkey serum, 0.1% Triton-X, 1% BSA and 0.05% sodium azide in PBS for 2 h, and then incubated with the primary antibodies overnight. This was followed by several washes and incubation with secondary antibodies for 2 h. Finally, samples were washed for 2 h with PBS and flat-mounted on glass slides with Mowiol. Z-stack images were acquired with a Zeiss LSM 710-FCS confocal microscope. Primary antibodies used were rabbit anti-mouse LYVE-1 (AngioBio, 1:600) and rat anti-mouse CD31 (BD Pharmingen, 1:250). Alexa 594- and 647-conjugated secondary antibodies were purchased from Invitrogen and used at a 1:200 dilution.

### 3T3-L1 adipogenic differentiation assays

Adipogenic differentiation assays were performed as previously described [1]. 3T3-L1 mouse embryonic fibroblasts (preadipocytes) were expanded in high-glucose (4.5 g/L) Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% bovine serum (Sigma) and penicillin/streptomycin. The cells were then plated onto collagen type I coated plates and were incubated in high-glucose DMEM, supplemented with 10% fetal bovine serum and penicillin/streptomycin (all from Gibco) in a humidified incubator at 5% $CO_2$. 48 hours post-confluence, adipogenic differentiation was induced with insulin (172 nM), dexamethasone (1 μM) and isobutyl-methylxanthine (115 μg/mL) (all from Sigma). To test if VEGF-C has an

effect on adipogenic differentiation of 3T3-L1 cells, 200 ng/mL human VEGF-C (kind gift from M. Jeltsch, University of Helsinki, Finland [2]) or an equivalent volume of PBS were added to the induction medium. Rosiglitazone (10 μM) was used as a positive control for the induction of adipogenic differentiation. After 2 days, the medium was changed to insulin medium, which contained only 172 nM insulin and 200 ng/mL VEGF-C or equivalent volume of PBS for the experimental conditions, which was applied every two days. For analyzing the effect of macrophage-secreted cytokines on adipogenic differentiation, the medium was supplemented with 10% macrophage conditioned medium using the same set up. At day 10, cells were washed twice with PBS, fixed with 4% PFA and stained with BODIPY 493/503, Syto60 and Hoechst (all from Invitrogen) to stain lipid droplets, cytosol and nuclei, respectively. The percentage of differentiated adipocytes was analyzed with CellProfiler software as previously described [3] and the adipogenic differentiation observed in induction medium was set to 100%.

**SVF differentiation assay**

SVF isolation, differentiation and quantification were performed as previously described [3, 4]. Briefly, SVF from mouse subcutaneous adipose tissue was isolated and plated on collagen-coated plates in 96-well format. The adipogenic differentiation was induced when cells reached 80% confluence and 200 ng/mL hVEGF-C (kind gift from M. Jeltsch, University of Helsinki, Finland [2]) or PBS in equivalent volume was applied on days 0, 2, 4 and 6 of differentiation. Thereafter, the cells were fixed and stained on day 8, and 9 images were acquired per well using an automated microscope system (CellWorks, Thermo Fisher). Images were analyzed as described above.

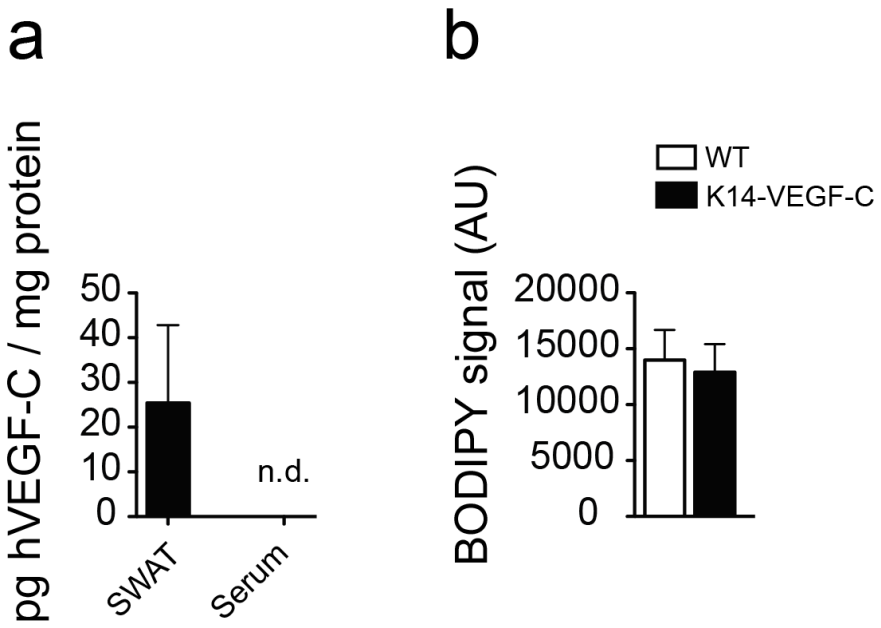
**Bomb calorimetry of stool samples**

Fecal energy content was measured by bomb calorimetry. Briefly, stool samples were collected from 17-week old mice that were on chow diet or HFD (n = 6-8 per group) and dried overnight at 37°C and the energy content of pooled samples from each genotype was analyzed by using an IKA Calorimeter (IKA-Werke GMBH).

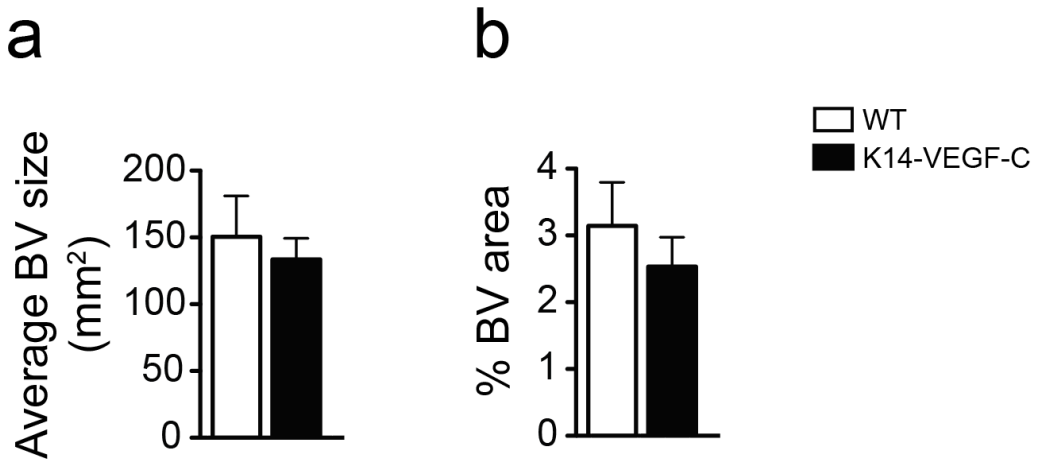
**Analysis of gene expression**

RNA was isolated from SWAT and EWAT samples using RNeasy Mini Kit (Qiagen) and from SWAT macrophages using RNeasy Micro Kit (Qiagen) following manufacturer's instructions. cDNA was transcribed from 1 μg of RNA using the High Capacity Reverse Transcription kit (Applied Biosystems). The PCR reactions were performed using FastStart SYBR green master mix (Roche) and a 7900HT Fast Real-Time PCR System (Applied Biosystems) using the following primer sets [1], *Vegfr3*-Forward: 5′- CTGGCAAATGGTTACTCCATGA-3′, *Vegfr3*-Reverse: 5′-ACAACCCGTGTGTCTTCACTG-3′, and *Rplp0* as internal control *Rplp0*-Forward: 5′-AGATTCGGGATATGCTGTTGGC-3′, *Rplp0*-Reverse: 5′-TCGGGTCCTAGACCAGTGTTC-3′ and gene expression fold changes were calculated using the $\Delta\Delta_{CT}$ method.

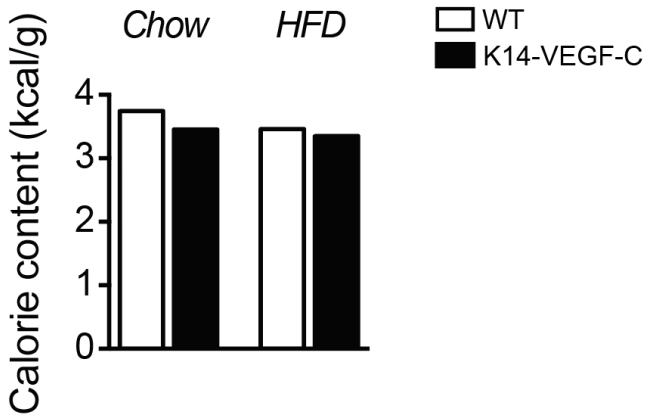
**SUPPLEMENTARY FIGURES and FIGURE LEGENDS**



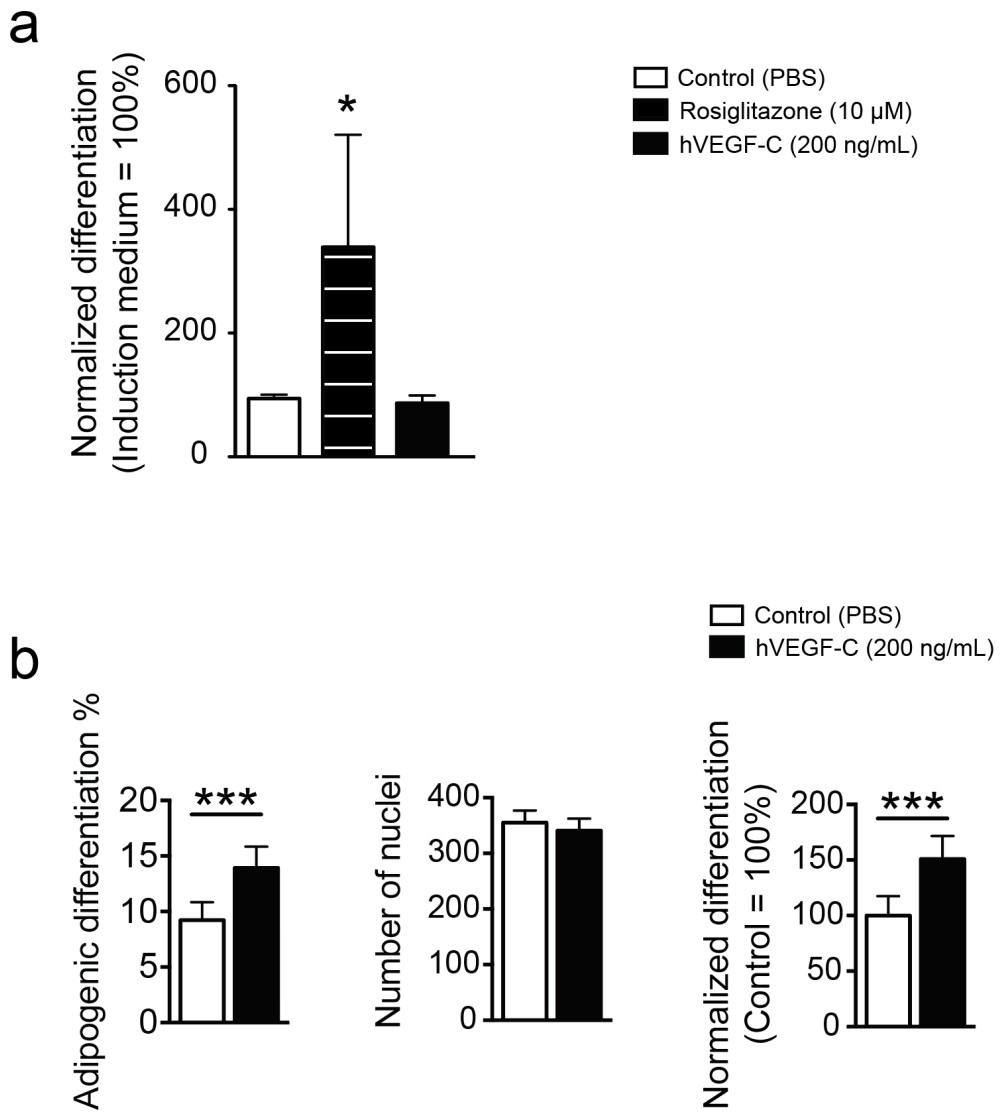

**Supplementary Figure S1: Overexpressed hVEGF-C could be detected in SWAT and does not affect lipid uptake from intestinal lacteals.** (**a**) ELISA analysis of SWAT and serum samples showing detectable amounts of hVEGF-C in SWAT (*n*=5 per group) but not in serum. Tissue samples from WT mice were used as controls as they do not contain hVEGF-C (not shown). n.d., not-detected. (**b**) BODIPY fluorescence measured in the serum 1 h after oral gavage revealed comparable lipid uptake in WT and K14-VEGF-C mice (*n*=3-4, two-tailed Student's *t*-test). Data are mean ± SD.



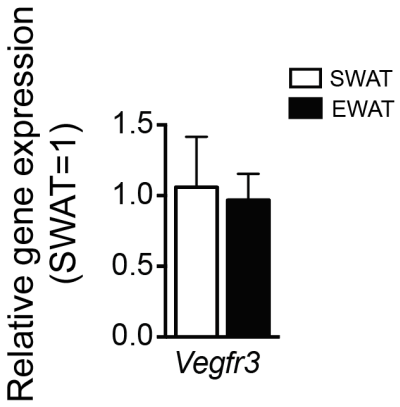

**Supplementary Figure S2: Blood vasculature in the skin is not affected by transgenic overexpression of hVEGF-C.** (**a**) Average blood vessel (BV) size and (**b**) % area covered by blood vessels (normalized to the entire region of interest) were measured from immunofluorescence stains of skin sections (shown in Figure 1B) of 20-week-old WT and K14-VEGF-C mice, revealing comparable blood vascularity at the site of transgenic overexpression ($n$=5 per group, two-tailed Student's $t$-test). Data are mean ± SD.




**Supplementary Figure S3: Energy content of stool samples.** Bomb calorimetry of fecal samples showed no major differences in the energy content. Data from pooled samples is shown (*n*=6 for WT groups and *n*=8 for K14-VEGF-C groups).




**Supplementary Figure S4: Effect of VEGF-C on adipogenic differentiation.** (**a**) No differences in adipogenic differentiation were detected when 3T3-L1 cells were treated with 200 ng/mL hVEGF-C, whereas the positive control Rosiglitazone (10 µM) promoted adipogenic differentiation. Results showing mean ± SD of three independent experiments. **p*<0.05, one-way ANOVA followed by Dunnett's multiple comparison post-hoc test. (**b**) On the other hand, 200 ng/mL hVEGF-C application induced adipogenic differentiation of SWAT stromal vascular fractions as compared to control without affecting cell proliferation (*n*=12 per group, two-tailed Student's *t*-test). **p*<0.0001. Data are mean ± SD.




**Supplementary Figure S5: *Vegfr3* gene expression in SWAT and EWAT.** No differences in *Vegfr3* gene expression were found between SWAT and EWAT under steady state conditions ($n$=8 per group). Data are mean ± SD.

## SUPPLEMENTARY REFERENCES


1. Karaman, S. *et al.* Blockade of VEGF-C and VEGF-D modulates adipose tissue inflammation and improves metabolic parameters under high-fat diet. *Mol Metab* **4**, 93–105 (2015).

2. Karpanen, T. *et al.* Lymphangiogenic growth factor responsiveness is modulated by postnatal lymphatic vessel maturation. *Am J Pathol* **169**, 708–718 (2006).

3. Meissburger, B. *et al.* Adipogenesis and insulin sensitivity in obesity are regulated by retinoid-related orphan receptor gamma. *EMBO Mol Med* **3**, 637–651 (2011).

4. Meissburger, B. *et al.* Tissue inhibitor of matrix metalloproteinase 1 (TIMP1) controls adipogenesis in obesity in mice and in humans. *Diabetologia* **54**, 1468-79 (2011).