Supplemental data

Maternal Retinoids Increase PDGFRα$^+$ Progenitor Population and Beige Adipogenesis in Progeny by Stimulating Vascular Development

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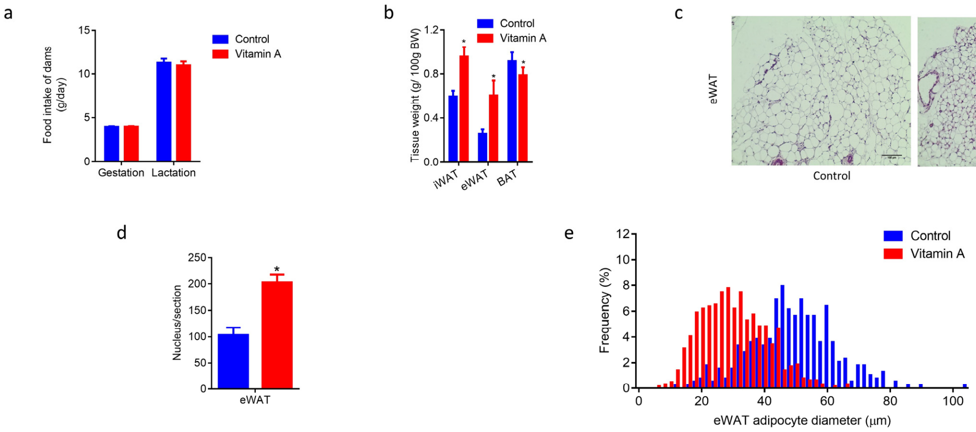

Figure S1. Maternal vitamin A supplementation affects adipose tissue deposition and morphology. For the MVA offspring, (**a**) Adipose tissue weight was calculated as the ratio to body weight. (**b**) Representative images of H&E stained eWAT. (**c**) Average number of nucleus per section. (**d**) Distribution of adipocytes size in eWAT. (**e**) Food intake of mother mice during pregnancy and lactation. Data presented are mean ± SEM, n = 6, unpaired two-tail t-test, *$p < 0.05$.

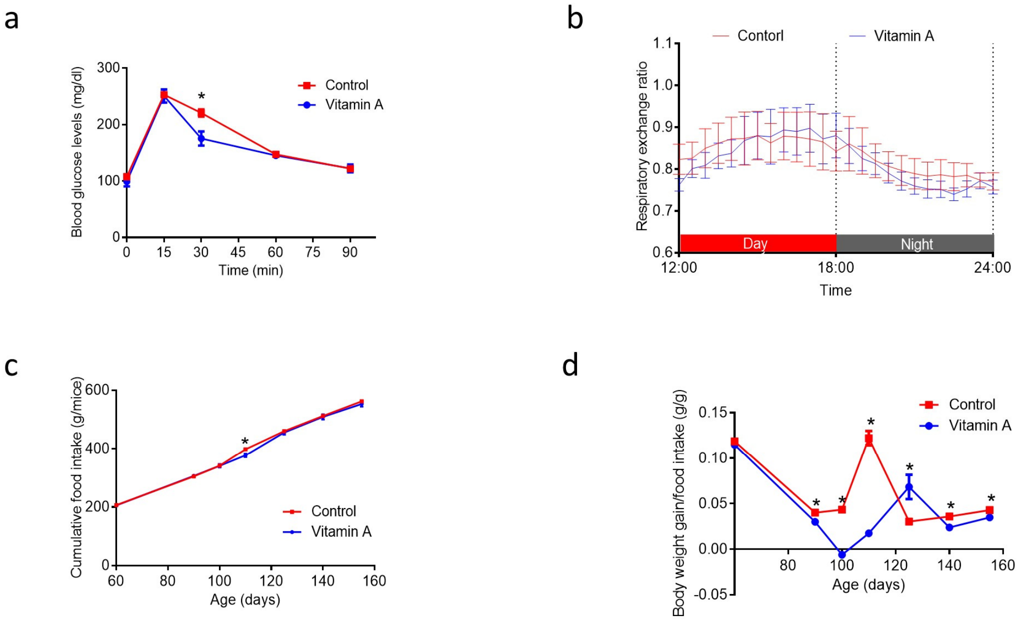

Figure S2. Maternal vitamin A supplementation prevents offspring obesity development induced by high fat diet. MVA offspring were fed a high fat diet (HFD, 45% energy from fat) from 30 days to 155 days old. (**a**) Glucose tolerance test at the beginning of HFD feeding (n = 3). (**b**) Respiratory exchange ratio after HFD feeding (n = 4). (**c**) Food intake during HFD feeding (n = 3). (**d**) Ratio of body weight gain/food intake (n = 3). Data presented are mean ± SEM, unpaired two-tail t-test, *$p < 0.05$.

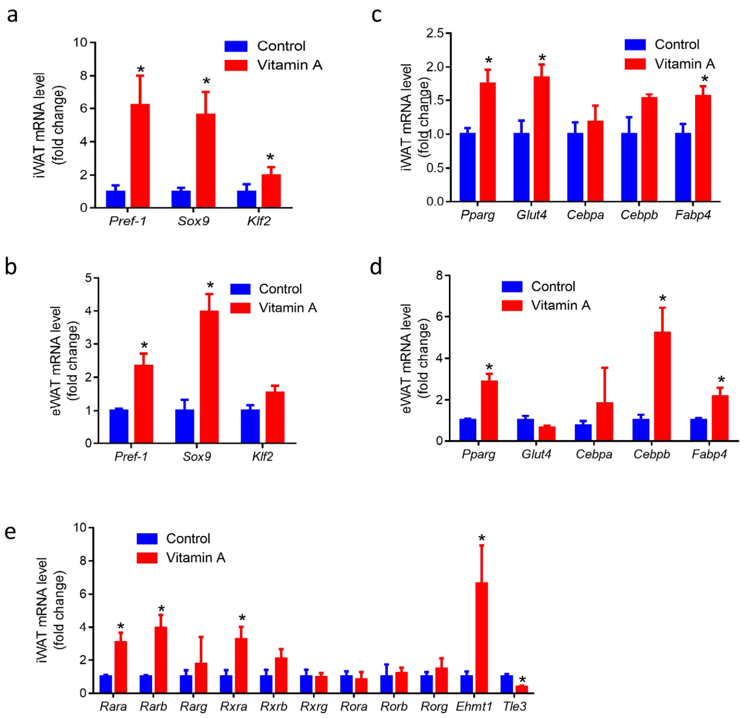

Figure S3. Maternal vitamin A supplementation promotes brown/beige adipogenesis in both white and brown adipose tissue. (**a-b**) mRNA levels of preadipose marker genes in iWAT (a) and eWAT (b). (**c-d**) mRNA levels of adipocyte markers in iWAT (c) and eWAT (d). (**e**) mRNA levels of RARs, RORs and other adipose related genes in iWAT. Data presented are mean ± SEM, n = 6, unpaired two-tail t-test, *$p < 0.05$.

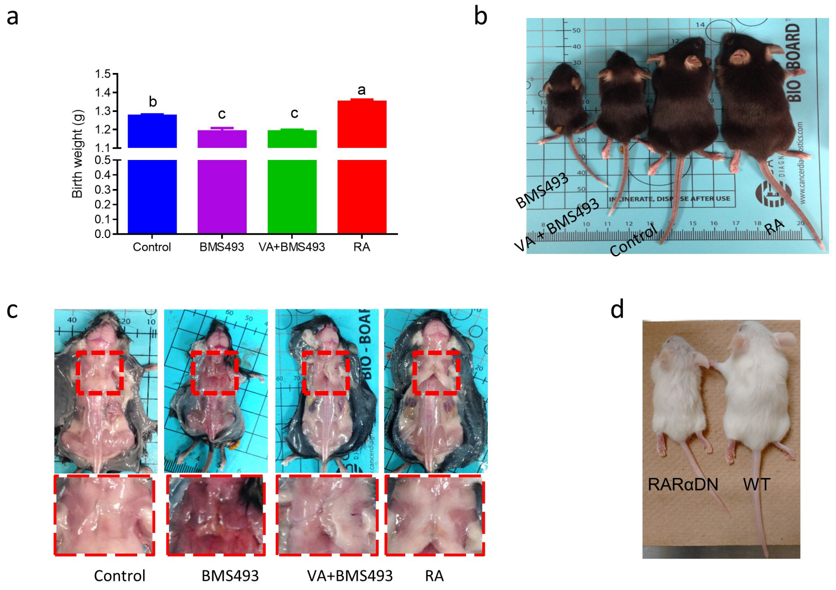

Figure S4. Retinoic acid signaling is required for fetal adipose tissue development. Pregnant mice were injected with BMS493 or RA at E10.5 and 13.5, and one BMS493 treated group was supplemented with vitamin A during gestation. (**a**) Birth weight. (**b**) Representative images of offspring at weaning (**c**) Representative images of subcutaneous fat tissue at weaning. (**d**) Representative images of weaning *Pdgfra*-cre/ER/RARαDN offspring. Data presented are mean ± SEM, n = 6, ANOVA, bars with different letters differ significantly.

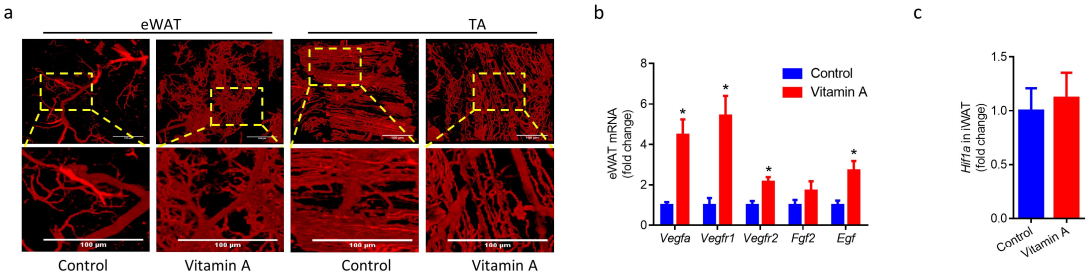

Figure S5. Maternal retinoic acid promotes vasculature in adipose tissues. For the MVA weanling offspring, (**a**) Blood vessels in eWAT and *Tibialis anterior* (TA) muscle stained by DiI. (**b**) mRNA levels of angiogenic genes in eWAT. (**c**) Expression of *Hif1a* in iWAT. n = 6. Data presented are mean ± SEM, unpaired two-tail t-test, *$p < 0.05$.

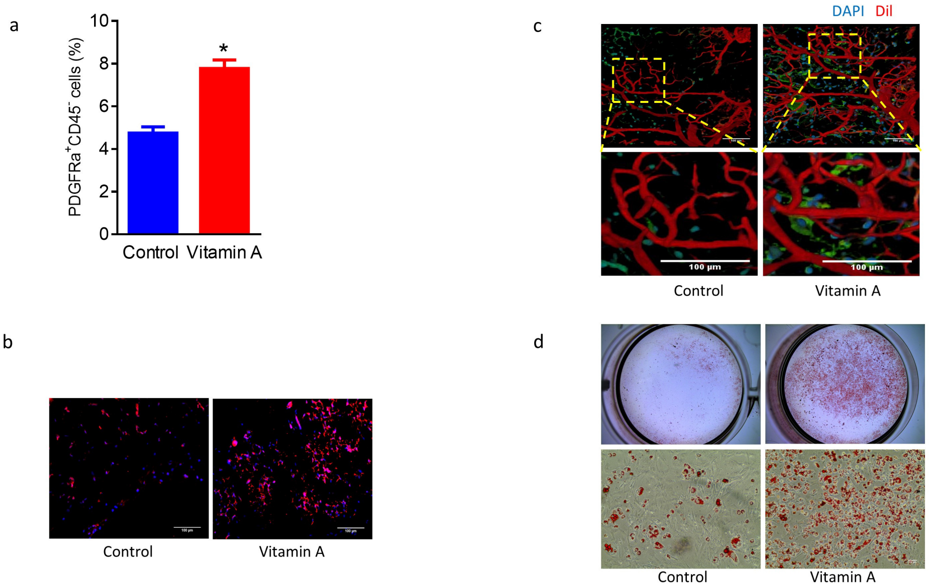

Figure S6. Maternal vitamin A increases PDGFRα$^+$ population in adipose tissue of weanling offspring. (**a**) Quantification of PDGFRα$^+$CD45$^-$ cells in iWAT analyzed by flow cytometry. (**b**) Images of IHC staining of eWAT section using anti-PDGFRα antibody. (**c**) Representative images showing blood vessels and PDGFRα$^+$ cells in iWAT. (**d**) iWAT derived SVCs differentiated for 6 days in brown adipogenic medium, and adipocytes were stained by Oil-Red O. Data presented are mean ± SEM, unpaired two-tail t-test, n = 4, *$p < 0.05$.

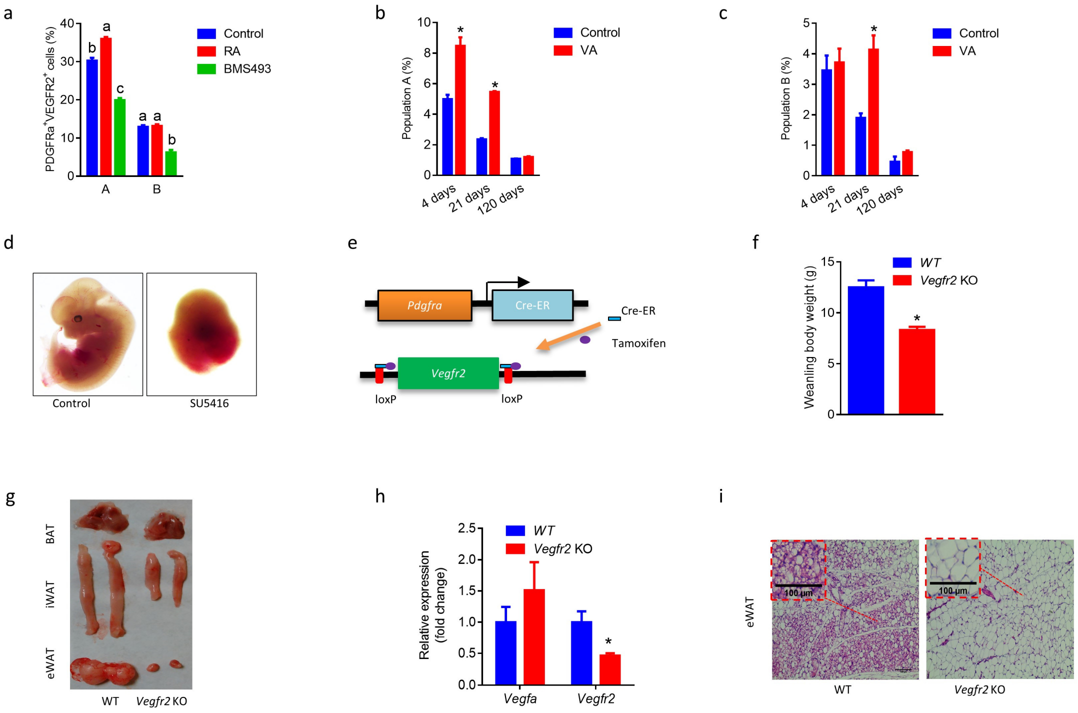

Figure S7. VEGF signaling plays an important role in RA regulation of adipogenesis. (a-c) Quantification of PDGFRα$^+$VEGFR2$^+$ cells in iWAT of RA, BMS493 injected (a) or MVA offspring at different ages (b and c) (n = 4). **(d)** Representative images showing E12.5 embryos which were prenatally treated with 10 mg/kg BW SU5416 at E10.5. (**e**-**i**) *Vegfr2* was knocked out in PDGFRα$^+$ cells at E10.5 by an injection of 20 mg/kg BW tamoxifen to the pregnant mothers (e). Body weight was measured at weaning (f, n = 6). Representative images of adipose tissue mass were showed (g). mRNA expression of *Vegfa* and *Vegfr2* was measured by qRT-PCR (h, n =6). eWAT sections were stained by H&E (i). Data presented are mean ± SEM, unpaired two-tail t-test (b-c, f and h) *p < 0.05 or ANOVA (a), bars with different letters differ significantly.

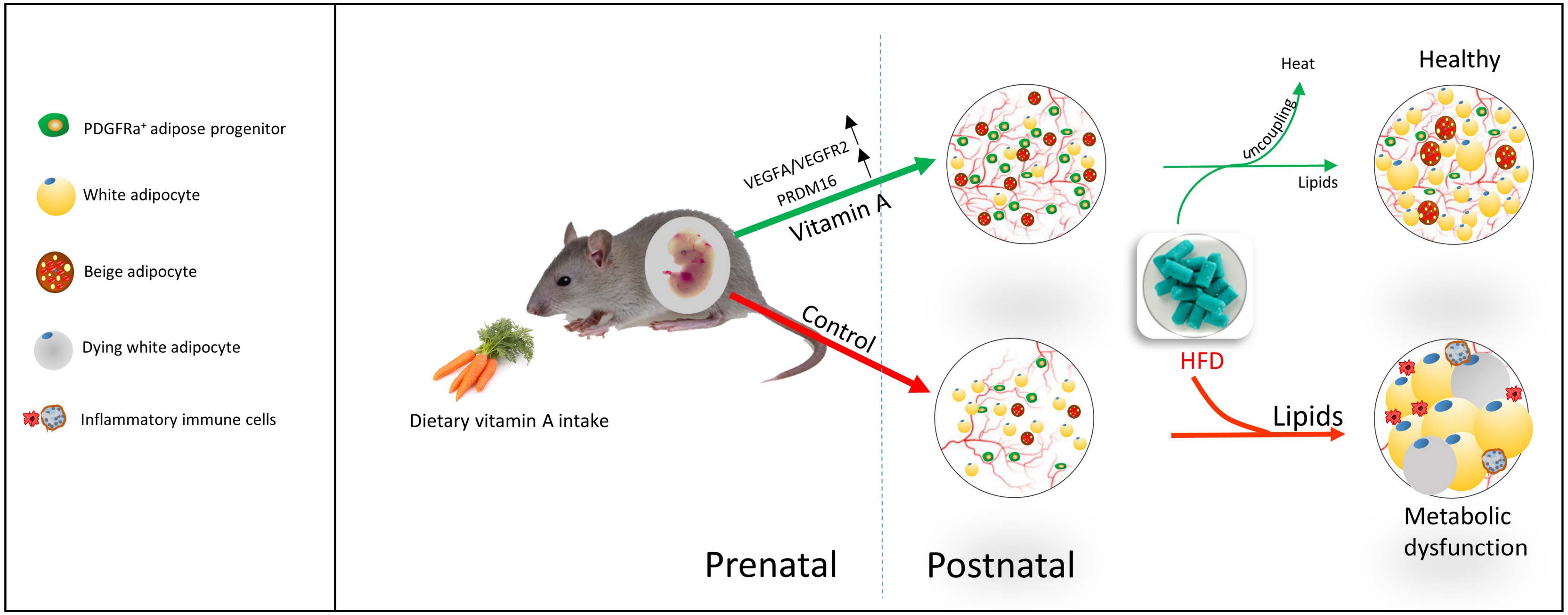

Figure S8. Diagram showing that maternal supplementation of vitamin A promotes angiogenesis and beige adipogenesis, which protects offspring from high fat-induced obesity.

Supplemental Table 1. qPCR primer sequences.

Gene name	Forward	Reverse	Product size (bp)
Zfp423	GTCACCAGTGCCCAGGAAGAAGAC	AACATCTGGTTGCACAGTTTACACTCAT	144
Pref1	TGACAATGTCTGCAGGTGCCAT	TATTTCGCAGAATTTCCCGTCC	145
Sox9	GGAAGTCGGTGAAGAACGGA	GGACCCTGAGATTGCCCAGA	166
Klf2	TAGTGGTGTGGCTGGGGATA	TTATGGCTCAAAGTAGCAGACT	178
Pparg	AGCTCCAAGAATACCAAAGTGCGAT	AGGTTCTTCATGAGGCCTGTTGTAGA	98
Cebpa	ATGAGAGAAGGAGGGGAGCAGG	AGGTGGGAGAGGCGTGGAACTA	203
Fabp4	CGACAGGAAGGTGAAGAGCATCATA	CATAAACTCTTGTGGAAGTCACGCCT	158
Glut4	CTCTCAGGCATCAATGCTGTTTTCTA	CGAGACCAACGTGAAGACCGTATT	123
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG	87
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG	123
Pdgfra	GGACTTACCCTGGAGAAGTGAGAA	ACACCAGTTTGATGGATGGGA	78
Vegfa	TGGACCCTGGCTTTACTGCT	GCAGTAGCTTCGCTGGTAGA	126
Vegfr1	GGCCCGGGATATTTATAAGAAC	CCATCCATTTTAGGGGAAGTC	71
Vegfr2	CAGTGGGATGGTCCTTGCAT	ACGGTGGTGTCTGTGTCATC	177
Egf	GTTAGCACCATCCCTCATCC	TCTGAGTGCAGCCGGAAAG	246
Fgf2	GGCTGCTGGCTTCTAAGTGT	GTCCCGTTTTGGATCCGAGT	153
Pgc1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC	161
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT	136
Elovl3	GATGGTTCTGGGCACCATCTT	CGTTGTTGTGTGGCATCCTT	73
Cox7a1	CAGCGTCATGGTCAGTCTGT	AGAAAACCGTGTGGCAGAGA	112
Rxra	TGGGGCTTGTGAGGTGTATTC	CACCTGGGTAGAGAAGTCGAAA	96
Rxrb	GCGTCCTTCTCCCATCGGTC	CAAAGATGGCTCCCACGCCT	109
Rxrg	GAACCACGCGCAACAGAAC	CTGCGTCTCCGTGACTAACA	81
Rara	TGGGGCGAGATGTACGAGA	CTCGATGGAGTGGTTTGAGC	186
Rarb	CAGTGCCCATACTGGTGTCT	AGGGCTGTTAATCCAGGAGG	186
Rarg	CTGCATGGAATCGTTTGTCCC	TCTCCACCGATTGTAGACTGG	152
Rora	CGCGGCGTAAAGGATGTATT	CCGACCAAACTTGACAGCATC	286
Rorb	TCTCCACAGCATTTGCCAGT	AAGGTGGTGAGTGTGTGCAA	299
Rorg	TGCTGTCCTGGGCTACCCTAC	CAAACTTGACAGCATCTCGGGAC	298
Tle3	CGGGCAAACCTCCAGGCAT	AGCTGGTGAGGGTGATGGGT	75
Ehmt1	GGCACCTTTGTCTGCGAATAC	AGAACCGAGCGTCAATGCAG	130
Hif1a	AGGATGAGTTCTGAACGTCGAA	AAACCATGTCGCCGTCATCT	298
18s	TTGTACACACCGCCCGTCGC	CTTCTCAGCGCTCCGCCAGG	102