Project Title: Energizing good fat by neurovascular development

Define the role of Ang-2 in VEGF-A mediated functional blood vessel formation in adipose tissue.

1). We have validated the overexpression of VEGF-A in Adipo-VEGF-A Tg mice. Overexpression of VEGF-A in sWAT increases ANG2 resulting in increase in vessel formation.

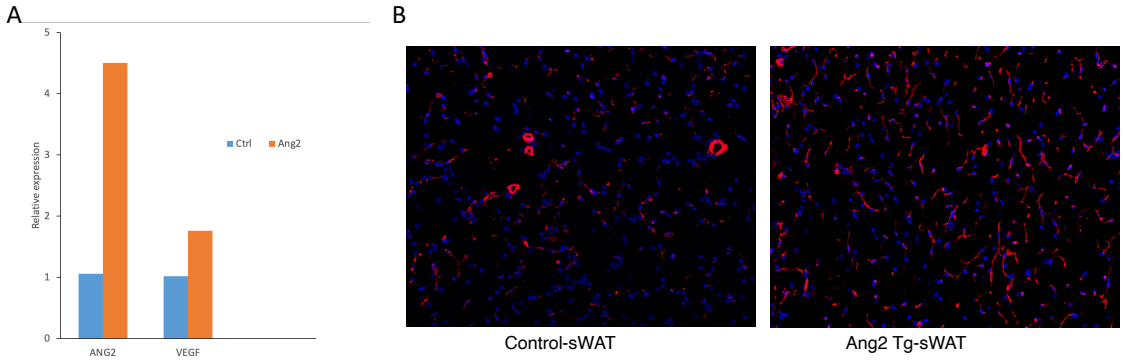


Fig 1. A) Q-PCR analysis of Ang-2 and VEGF-A in subcutaneous white adipose tissue (sWAT). B) IF staining with anti-endomucin (red), marker for blood vessel in sWAT.

2). Adipose tissue-derived VEGF-A induces angiogenesis and nerve innervation.

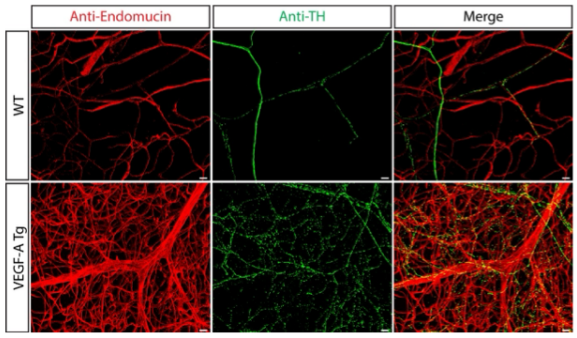


Fig. 2. Whole mount immunofluorescent staining by anti-endomucin antibody (red, the marker of blood vessels) and anti-tyrosine hydroxylase (TH) antibody (green, the marker of nerve fibers) in subcutaneous adipose tissue of VEGF-A transgenic mouse (bottom panel) and its littermate control (upper panel).

Investigate the function of sympathetic innervation on VEGF-A mediated angiogenesis.

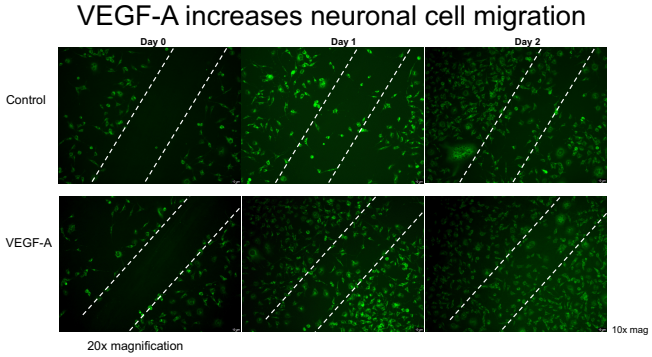


Fig. 3. Cell migration scratch assay. Left most panel is day 0 where there is a clear scratch and no cells. On Day 1 which is 24 hours later cells start to migrate to the area of the scratch and by day 2 this is almost completely filled up for the VEGF treated cells suggesting that VEGF increases cell migration SH-SY5Y neuronal cells.

scRNA-seq data set

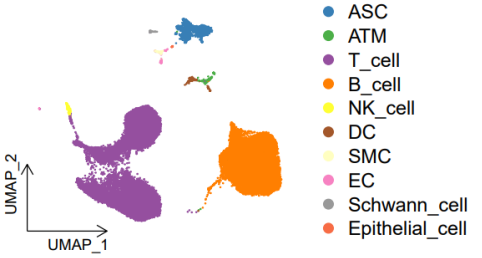
Introduction of dataset:
The scRNA-seq data is derived from four groups of samples, with one sample from each group.
1.SAT Tg VEGF: This group consists of mice treated with VEGF, with sample taken from subcutaneous adipose tissue (SAT).
2.SAT ctl: This group consists of control mice treated with no VEGF, with sample taken from subcutaneous adipose tissue (SAT).
3.IP Tg VEGF: This group consists of mice treated with VEGF, with sample collected from visceral adipose tissue (IP).
4.IP ctl: This group consists of control mice treated with no VEGF, with sample taken from visceral adipose tissue (IP).

Methods:
The raw data was processed using CellRanger version 8.0.1 to generate the gene-cell unique molecular identifier (UMI) matrix, using the reference genome GRCm39. The UMI matrix was read using the read_10x_mtx function in Scanpy version 1.11.0rc2. Doublets were identified and removed using Scrublet version 0.2.3. Low-quality cells were excluded (> 40,000 UMI/cell, < 500 genes/cell, > 5,000 genes/cell, and > 15% mitochondrial genes). Batch effect correction was performed using Harmony version 1.2.3. Dimensionality reduction and unsupervised clustering were carried out using Seurat version 4.4.0. Differential cell-cell communication across groups was analyzed using CellChat version 2.1.2.

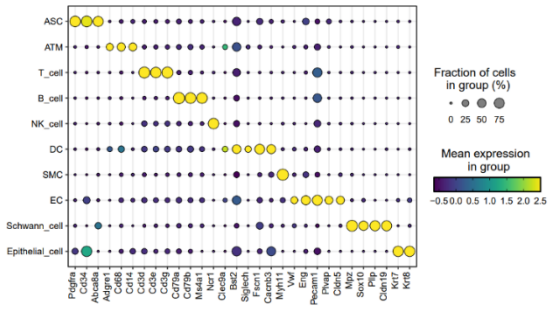
Quality control:

	IP_VEGF	IP_ctl	SAT_VEGF	SAT_ctl
cell_count	11426	11857	11060	10282
mean_nCount_RNA	5550.589	5752.644	5534.485	4850.78
median_nCount_RNA	4812	4985	4605.5	4231.5
max_nCount_RNA	34903	39188	33608	33251
min_nCount_RNA	704	673	749	730
mean_nFeature_RNA	1932.079	1997.8	1907.176	1749.508
median_nFeature_RNA	1810	1874	1753.5	1634
max_nFeature_RNA	4989	4997	4995	4989
min_nFeature_RNA	501	509	505	507
mean_percent_mt	3.95325	4.159302	4.184971	3.571314
median_percent_mt	3.824645	4.011503	4.058108	3.484497
max_percent_mt	14.84375	14.94075	14.86526	14.96015
min_percent_mt	0	0	0	0

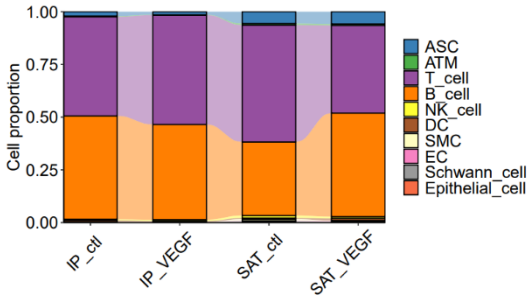
Results:



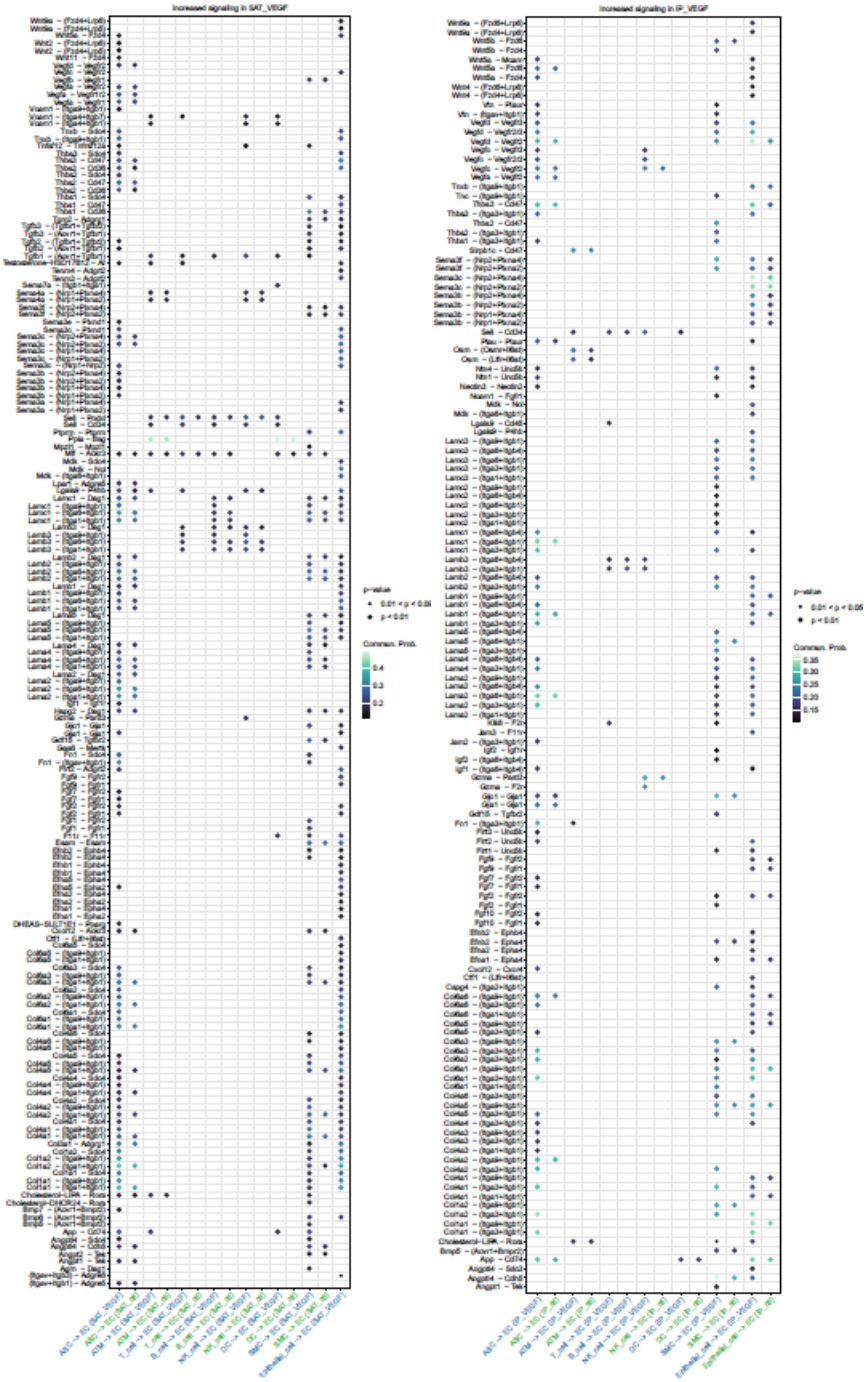
In the data, we cell types classical



scRNA-seq identified 10 based on marker genes, tissue including adipose stem cell (ASC), adipose macrophage (ATM), T cell, B cell, NK cell, dendritic cell (DC), smooth muscle cell (SMC), endothelial cell (EC), schwann cell, and epithelial cell.



The left panel shows the cell composition for each sample.



We compared the upregulated cell-cell communications between other cells and endothelial cells in SAT_VEGF vs SAT_ctl (left panel) and IP_VEGF vs IP_ctl (right panel) using CellChat. We found that VEGF can promote angiogenesis in endothelial cells by stimulating certain cell types to secrete pro-angiogenic factors. For example, VEGF may promote ASC cells to secrete Fgf2, which then acts on endothelial cells to promote angiogenesis.