Endothelial tip cells *in vitro* are less glycolytic and have a more flexible response to metabolic stress than non-tip cells

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Supplementary information

Supplementary Table 1. Primer details. Gene symbols, primer sequences, predicted size (bp) and melting temperature (Tm) of the amplification products for human genes tested.

Gene	GenBank	Forward primer	Reverse primer	Size(bp)	Tm (°C)
ANGPT2	NM_001147	GCAAAATAAGCAGCATCAGCCAAC	GCATCAAACCACCAGCCTCCT	115	76
CD34	NM_001025109	GGAGCAGGCTGATGCTGATG	ATCCCCAGCTTTTTCAGGTCAGAT	165	82
CXCR4	NM_003467.2	CGGTTACCATGGAGGGGATCAGTATATA	GCATTTTCTTCACGGAAACAGGGTTC	109	78
DLL4	NM_019074	AACGGGGGACAGTGCCTGAA	TGAGCCCATTCTCCAGGTCAT	151	86
IGF2	NM_000612.4	CCTCGTGCTGCATTGCTGCT	CTTGCGGGCCTGCTGAAGTAGAA	115	86
NRP2	NM_003872	GGAGCCCTGTGGTTGGATGTATG	TCATCTGGAAACGTCCGGTCGT	93	83
VEGFR2	NM_002253.2	CCAGATGACAACCAGACGGACAG	GGCACCATTCCACCAAAAGATG	104	77
VEGFR3	NM_002020	GCCAGGTATTACAACTGGGTGTCCT	TCTGGTTGTCCACAGAGCCTTTGT	136	83
YWHAZ	NM_003406	ACTTTTGGTACATTGTGGCTTCAA	CCGCCAGGACAAACCAGTAT	94	77

Supplementary Table 2. Primer details. Gene symbols, primer sequences, and location of human genes tested to determine mitochondrial DNA and nuclear DNA content.

Gene	GenBank	Forward primer	Reverse primer	Cellular location
COX2	NC_011137.1	GATCCCTCCCTTACCATCAAA	GCCGTAGTCGGTGTACTCGT	Mitochondrial DNA
COX3	NC_012920.1	TCCAAACATCACTTTGGCTTC	AACCACATCTACAAAATGCCAGT	Mitochondrial DNA
CYTB	NC_011137.1	TCATTATTGCAGCCCTAGCAG	GTTGTTTGATCCCGTTTCGT	Mitochondrial DNA
NCOA3	NC_000020.11	CCTCTGGGCTTTTATTGCGAC	CGGTCATCAGAAGAACAGGTAAGT	Nuclear DNA
B2M	NC_000015.10	AAGTTCGCATGTCCTAGCACC	TCACAGCCAAGCATTCTACAAAC	Nuclear DNA

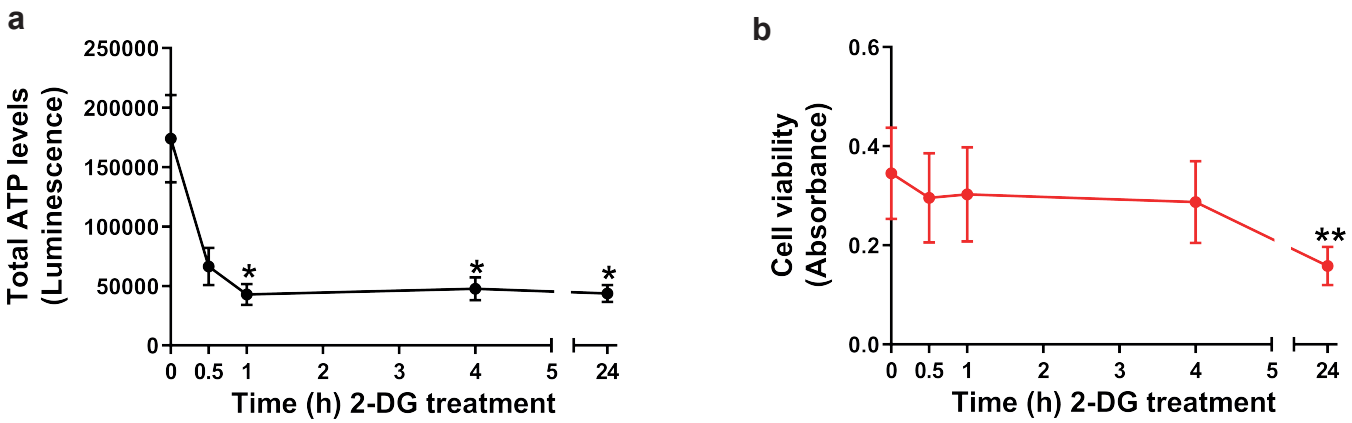

Fig. S1 Effects of blocking cellular glucose uptake on energy production and survival of HUVECs and on tip cell differentiation.

a HUVECs in culture were exposed to 100 mM 2-DG for 0.5, 1, 4, or 24 h and showed rapid depletion of total ATP levels during the first hour of treatment in HUVECs (determined as relative luminescence). Total ATP levels remained low up to 24 h of exposure. **b** HUVECs treated with 2-DG showed a significantly reduced viability (measured as absorbance) in the MTT assay after 24 h of treatment. Results are shown as means ± SEM of experiments with HUVECs of at least 3 donors. * $P<0.05$ and ** $P<0.01$ as compared to control (Unpaired Student's t-test).

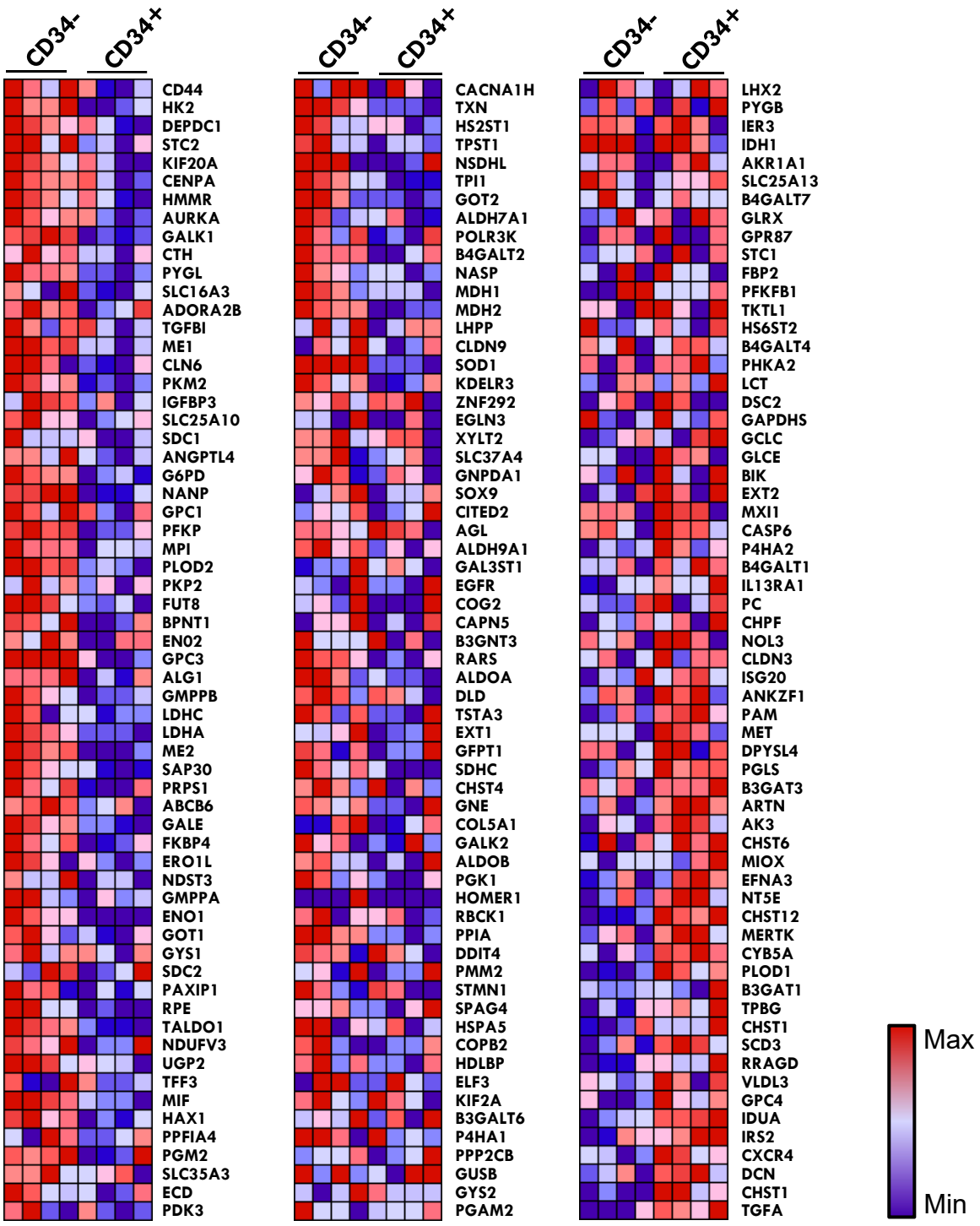

Fig. S2 Heat map differentially expressed genes involved in the glycolytic pathway in non-tip cells and tip cells. Heat map of the accompanying GSEA enrichment plot (Fig. 4d) for the glycolysis gene set in $CD34^-$ non-tip cells and $CD34^+$ tip cells are shown (N=4).

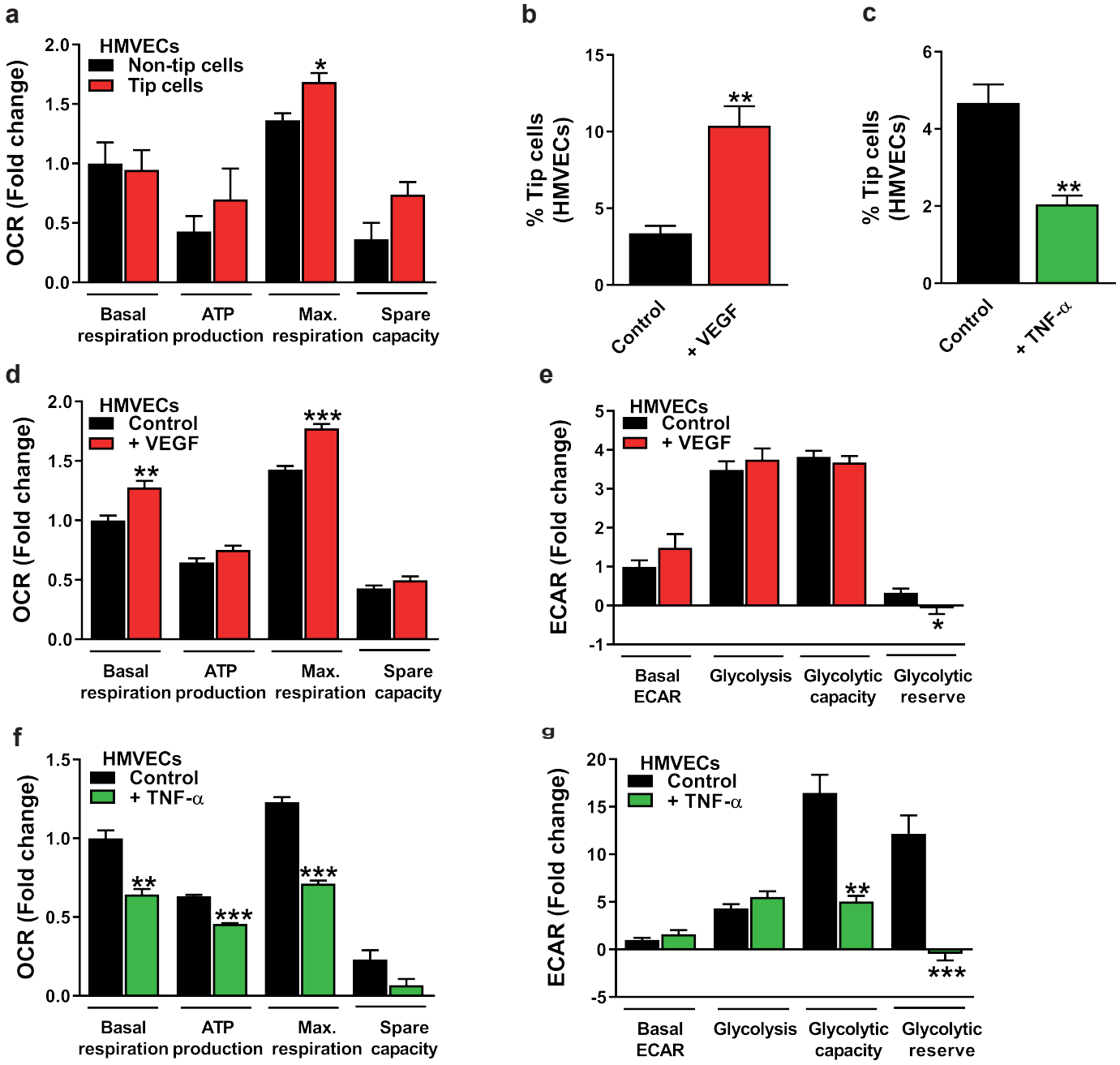

Fig. S3 Mitochondrial respiration and glycolysis in hMVECs.
HMVEC cells were treated with VEGF (25 ng/ml) or TNF-α (10 ng/ml) for 24 h. **a** FACS-sorted hMVECs showed a higher maximal respiration capacity and spare capacity in tip cells as compared to non-tip cells. Treatment of hMVEC cultures with VEGF induced the percentage of tip cells (**b**), whereas TNF-α reduced the percentage of tip cells (**c**). VEGF induced mitochondrial respiration (**d**) and reduced glycolytic reserve (**e**). TNF-α reduced mitochondrial respiration (**f**) and glycolytic capacity and glycolytic reserve (**g**). OCR and ECAR measurements were represented as fold change compared to control basal OCR and ECAR levels, respectively. Results are shown as means ± SEM of experiments with hMVECs of at least 3 donors. * P<0.05, **bP<0.01, and *** P<0.001 as compared to control (Unpaired Student's t-test).

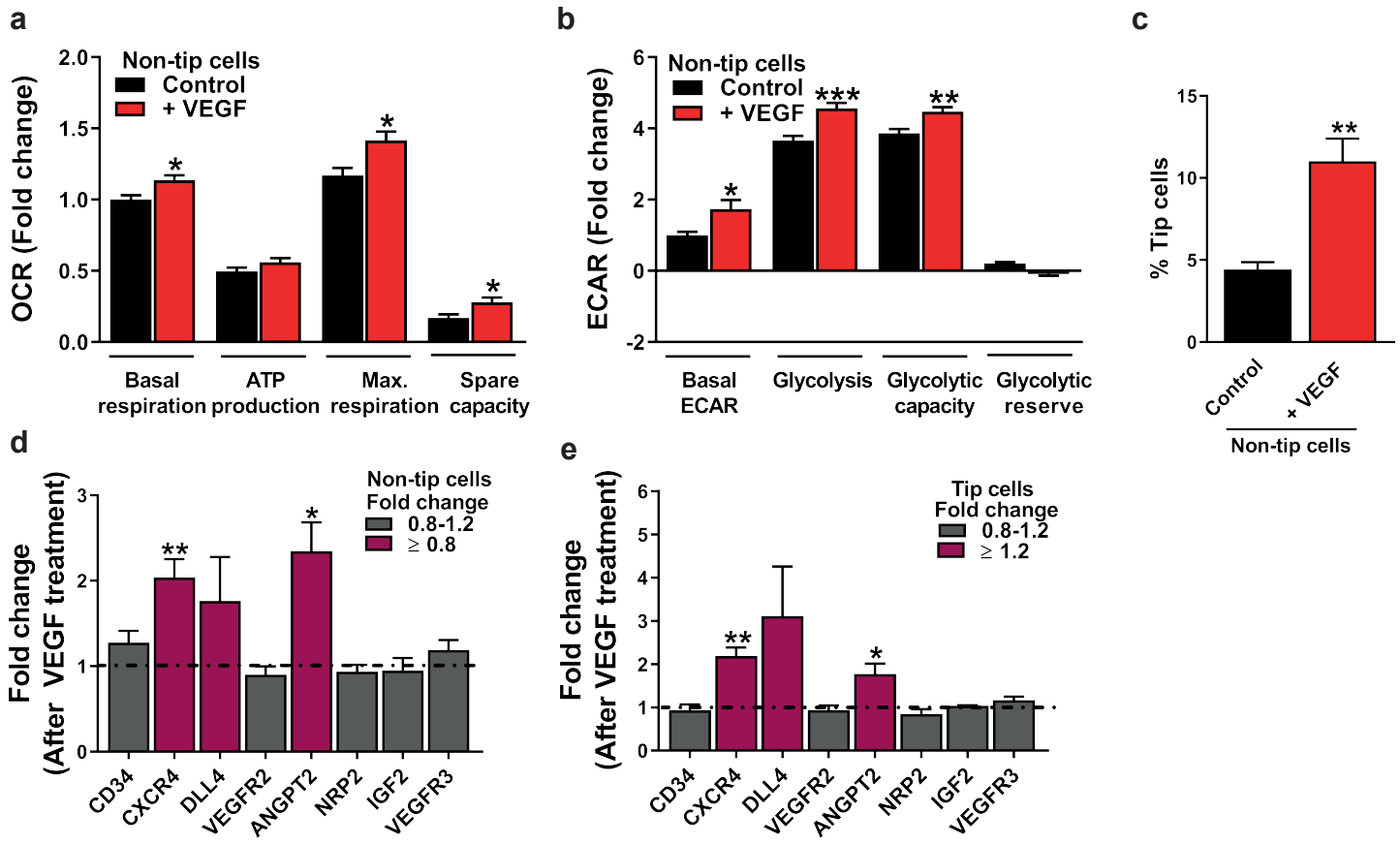

Fig. S4 Effects of VEGF on mitochondrial respiration, glycolysis and tip cell differentiation.
HUVECs were treated with VEGF (25 ng/ml) for 24 h. VEGF induced mitochondrial respiration (**a**) and glycolysis (**b**) in non-tip cells. Non-tip cells treated with VEGF showed induced percentages of tip cells (**c**) and induced mRNA expression levels of CXCR4, DLL4, and ANGPT2 (**d**). **e** Tip cells treated with VEGF did not show reduced mRNA expression levels of tip cell-specific genes. OCR and ECAR measurements were represented as fold change compared to control basal OCR and ECAR levels, respectively. Results are shown as means ± SEM of experiments with HUVECs of at least 3 donors. * P<0.05, ** P<0.01, and *** P<0.001 as compared to control (Unpaired Student's t-test).

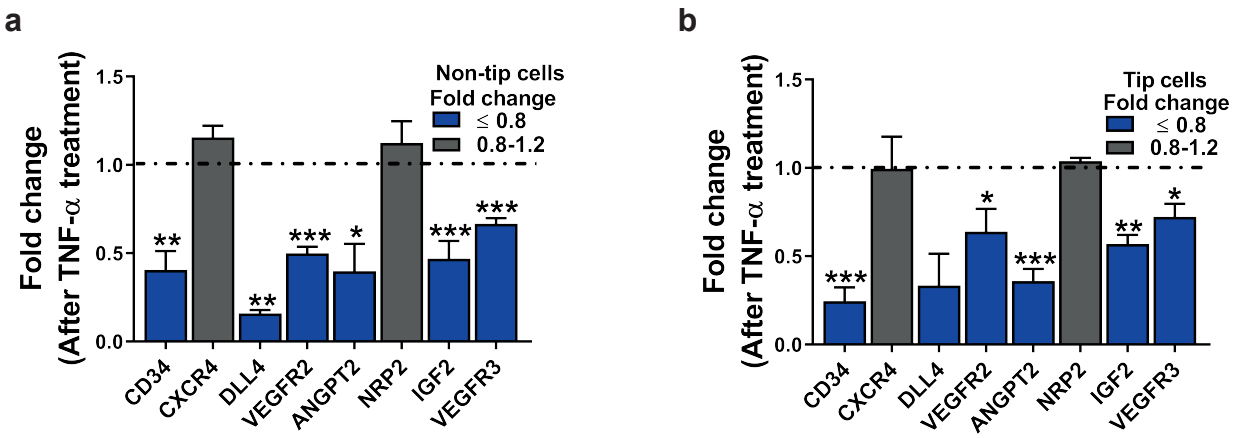

Fig. S5 Effects of TNF-α on mRNA expression levels of tip cell-specific genes.
Treatment of FACS-sorted HUVECs with TNF-α (10 ng/ml) reduced mRNA expression levels of 6 out of 8 tip cell-specific genes in non-tip cells (**a**) and reduced mRNA expression levels of 5 out of 8 tip cell-specific genes in tip cells (**b**) at 24 h after treatment. Results are shown as means ± SEM of experiments with HUVECs of at least 3 donors. * P<0.05, ** P<0.01, and *** P<0.001 as compared to control (Unpaired Student's t-test).

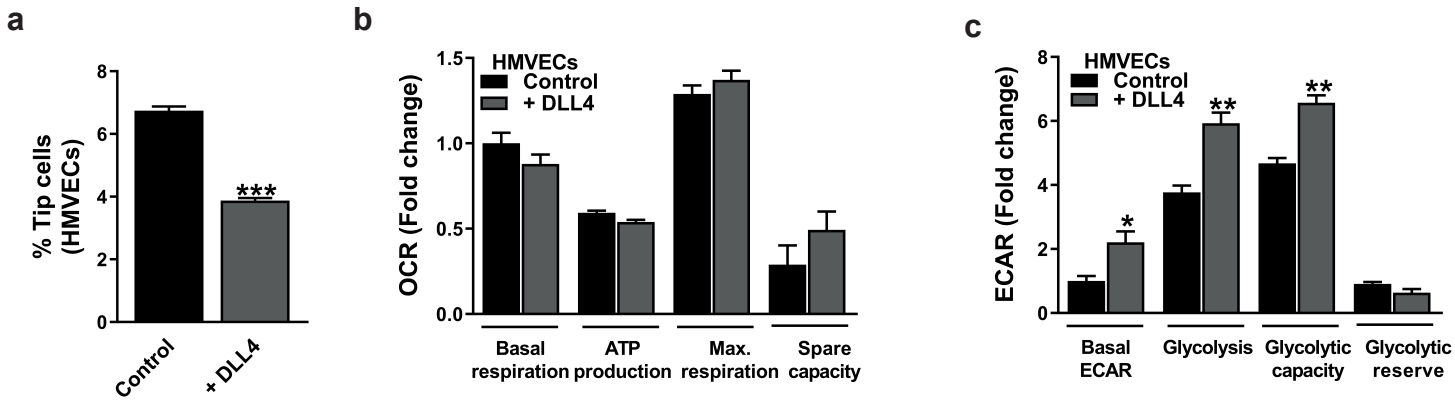

Fig. S6 Effects of DLL4 coating on mitochondrial respiration and glycolysis in hMVECs.
a HMVECs cultured on DLL4-coated (1µg/ml) plates showed lower tip cell percentages compared to BSA-coated (1 µg/ml) plates used as a control. DLL4 treatment did not affect mitochondrial respiration (**b**), but increased glycolysis (**c**) in hMVEC cultures 24 h after cell addition. OCR and ECAR measurements were represented as fold change compared to control basal OCR and ECAR levels, respectively. Results are shown as means ± SEM of experiments with HUVECs of at least 3 donors. * P<0.05 and ** P<0.01 as compared to control (Unpaired Student's t-test).