

Supplementary materials

Reagents

Reagent	brand	code	Application
Recombinant human TNFSF14/LIGHT	R&D systems	#664-LI-025/CF	Cell stimulation
NIK-SMI1	ProbeChem	PC-62514	Cell treatment
BAY11-7082	Cayman chem	10010266	Cell treatment
HiPerfect	Qiagen	301705	Cell transfection
Smooth muscle cell medium (SMCM)	ScienCell	1101-b	Cell culture
Smooth muscle cell growth supplements (SMCGS)	ScienCell	1152	Cell culture
Dulbecco's modified essential medium	Gibco	11995-065	Cell culture
Opti-MEM® I	Gibco	31985-070	Transfection
Penicillin/Streptomycin	ScienCell	0503	Cell culture
Foetal Bovine Serum	ScienCell	0010	Cell culture

Short interfering RNAs and plasmids

Reagent	Brand	Code	Dose used
siNTC	Dharmacon	D-001810-10-05	40nM
siHVEM-B	Origene	SR305773B	40nM
siLTβR	Dharmacon	L-008023-00-0005	25nM
pCMV6-XL4-HVEM	Origene	SC117733	20ng/ml
pCMV6-XL4	Origene	pCMV6-XL4	20ng/ml

Patient details

Patient	Gender	Age	Eosinophil count d/m/p	Use
1	M	13	40/70/>100	Fibroblasts
2	M	10	8/0/0	Fibroblasts
3	M	15	>100/75/6	Fibroblasts
4	M	12	0/0/0	Fibroblasts
5	M	5	3/0/0	Fibroblasts
6	F	2	0/0/0	Fibroblasts
7	F	19	100/30/45	Fibroblasts
8	M	16	100/100/72	Fibroblasts
9	Unknown	9	1/2/1	Fibroblasts
10	F	11	3/0/0	Fibroblasts
11	M	12	10/100/100	Fibroblasts
12	M	18	30/55/20	Fibroblasts/histology
13	M	8	27/2/0	Histology
14	F	9	30/0/0	Histology
15	M	7	85/10/85	Histology
16	M	10	>100/>100/>100	Histology
17	M	4	22/23/44	Histology
18	M	11	44/53/60	Histology
19	M	10	60/1/0	Histology

Primers

Gene	Primer	5'-3'
RPL13A	FWD	CATAGGAAGCTGGGAGCAAG
	REV	GCCCTCCAATCAGTCTTCTG
ICAM1	FWD	TTGTTGGGCATAGAGACCCC
	REV	GGTTTTAGCTGTTGACTGCCC
IL32	FWD	AGAGCTGGAGGACGACTTCA
	REV	CTCGGCACCGTAATCCATCT

IL33	FWD	CATCTGGTACTCGCTGCCTGTC
	REV	CAACACCGTCACCTGATTCATT
IL34	FWD	CTTGGGATCTTCCTTGGCGT
	REV	CAGCTTGTCCCGCAGAAAAC
CXCL5	FWD	CACGCAAGGAGTTCATCCCA
	REV	TCCTTCCCGTTCTTCAGGGA
BIRC3	FWD	TCATCCGTCAAGTTCAAGCCA
	REV	TCTCCTGGGCTGTCTGATGT
DKK1	FWD	TGACAACTACCAGCCGTACC
	REV	CGAGACAGATTTGCACGCCT
WNT2b	FWD	GATCCGAGAGTGTCAGCACC
	REV	CTGCCTCTCGGCTACTTCTG
WNT5a	FWD	TTTGGCAGGGTGATGCAGAT
	REV	GCCATAGTCGATGTTGTTCGC
BMP6	FWD	TCATTGCACCCAAGGGCTAT
	REV	GCACGGTTTGGGGACATACT
SEMA3B	FWD	CCTCCCTCAACCTGGACAAC
	REV	GCAAATGGGTGCGGTTGTAG
HVEM	FWD	GACCCTGGAGGAATGTCAGC
	REV	CGAGGCTCCCTGAGAGAAAC
LTβR	FWD	CCGACACAACCTGCAAAAAT
	REV	GAGCAGAAAGAAGGCCAGTG

Antibodies

Reagent	brand	code	Application
Anti-ICAM1-PE	eBioscience	#12-0549-42	Flow cytometry
Anti-VCAM1-APC	Biolegend	305810	Flow cytometry
Anti-CD74-PerCP	Biolegend	357607	Flow cytometry
Anti-HVEM-APC	Biolegend	318808	Flow cytometry
Polyclonal goat anti-LTβR	R&D systems	AF629	Flow cytometry
Anti-p65	Cell signaling technology	8242S	Western blot and immunofluorescence
Anti-p100/p52	Cell signaling technology	3017T	Western blot
Anti-GAPDH	Cell signaling technology	5174S	Western blot
Anti-Lamin	Thermo	MA3-1000	Western blot
Anti-β-Tubulin	Cell signaling technology	2128S	Western blot
Anti-Vimentin	Sigma	V6389	immunofluorescence
Anti-WNT2b	Abcam	Ab178418	immunofluorescence
Donkey anti-goat IgG Alexa Fluor 488	Life technologies	#A11055	Flow cytometry
Alexa fluor 488	Goat anti-rabbit IgG	Life technologies #A11008	ICC-F
Alexa fluor 488	Goat anti-mouse	Invitrogene, #A10680	IF-P
Alexa fluor 594	Goat Anti-rabbit	Life technologies, #A11012	IF-P
Isotype	Goat IgG control	R&D systems, #AB-108-C	Flow cytometry

Isotype-PE	Mouse IgG1k	eBioscience, #12-4714-82	Flow cytometry
Isotype-APC	Mouse IgG1	Biolegend, #406609	Flow cytometry
Isotype	Mouse IgG	Abcam, #ab190475	IF-P
Isotype	Rabbit polyclonal IgG	Abcam, #ab27478	IF-P
Phalloidin iFluor 594		ab176757	IF-C

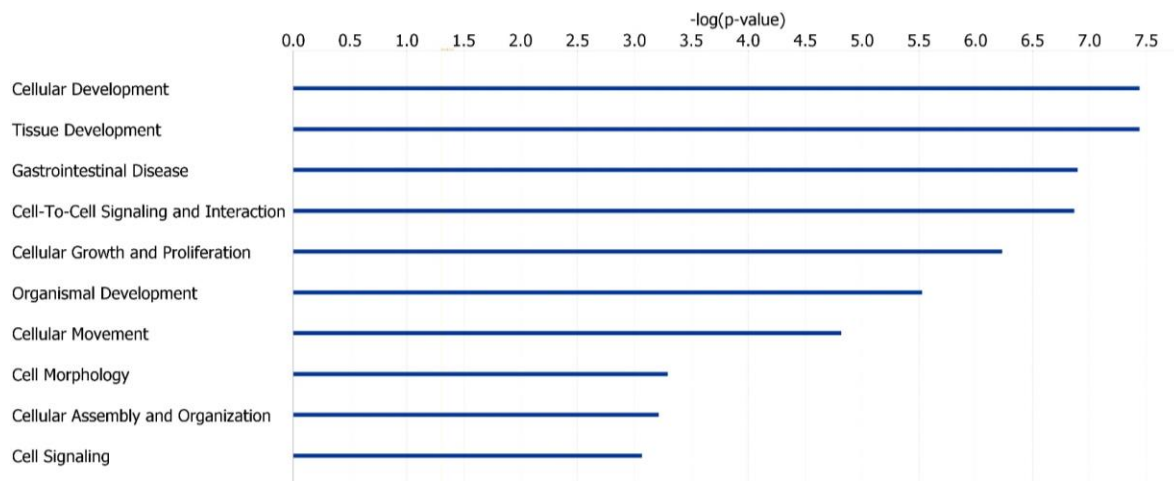
RNAscope probes

Reagent	Brand	code	Dilution
Hs-vimentin	ACD-Biotechne	310441-C2	1:50
Hs-ICAM1	ACD-Biotechne	402951	Undiluted
Hs-IL-34	ACD-Biotechne	313011-C3	1:50
Hs-WNT2B	ACD-Biotechne	453361	Undiluted

Supplementary figure 1

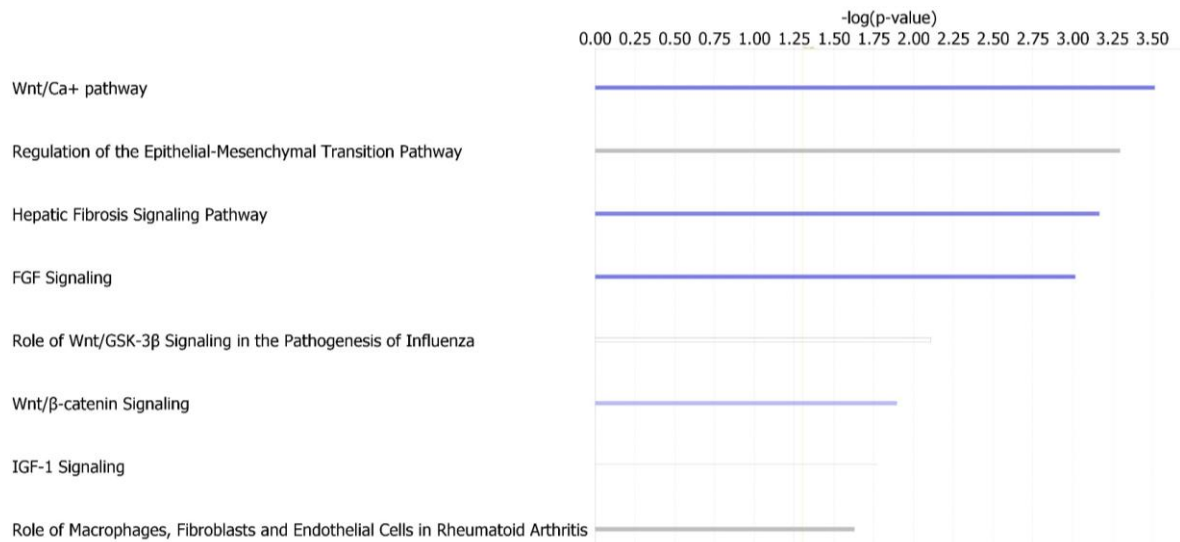
A

Diseases and functions

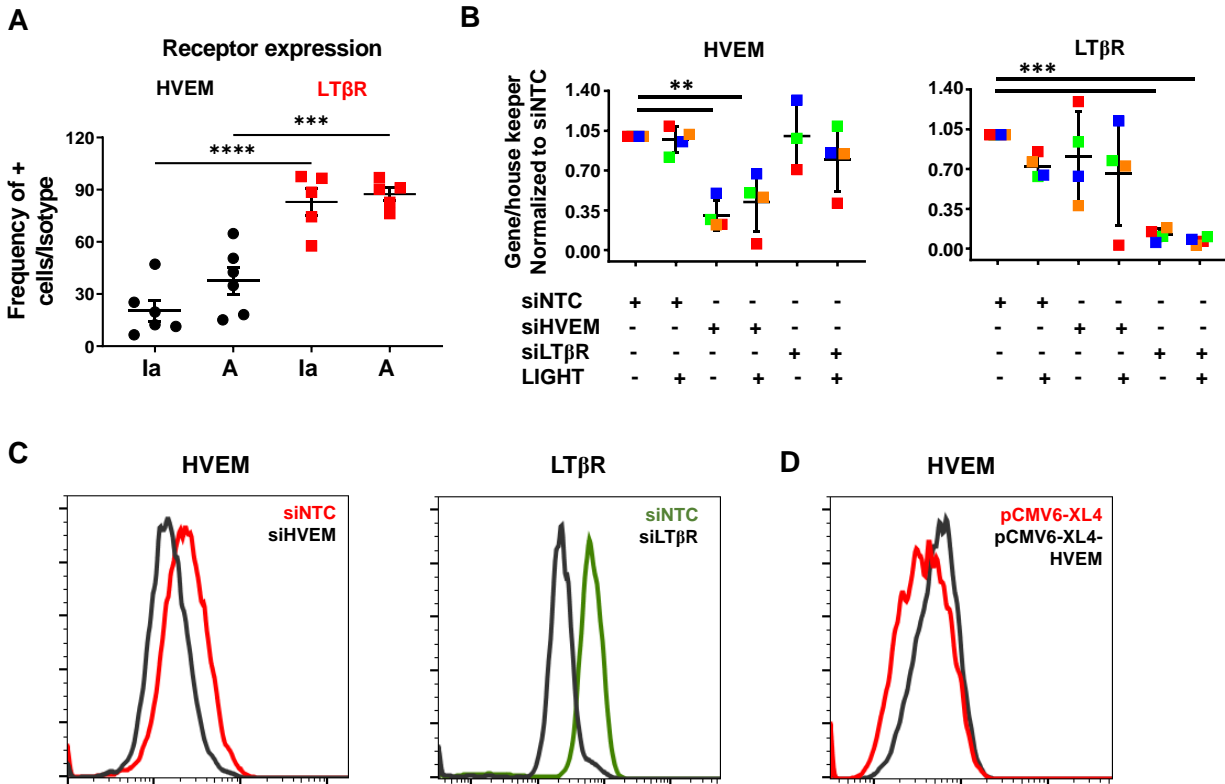


B

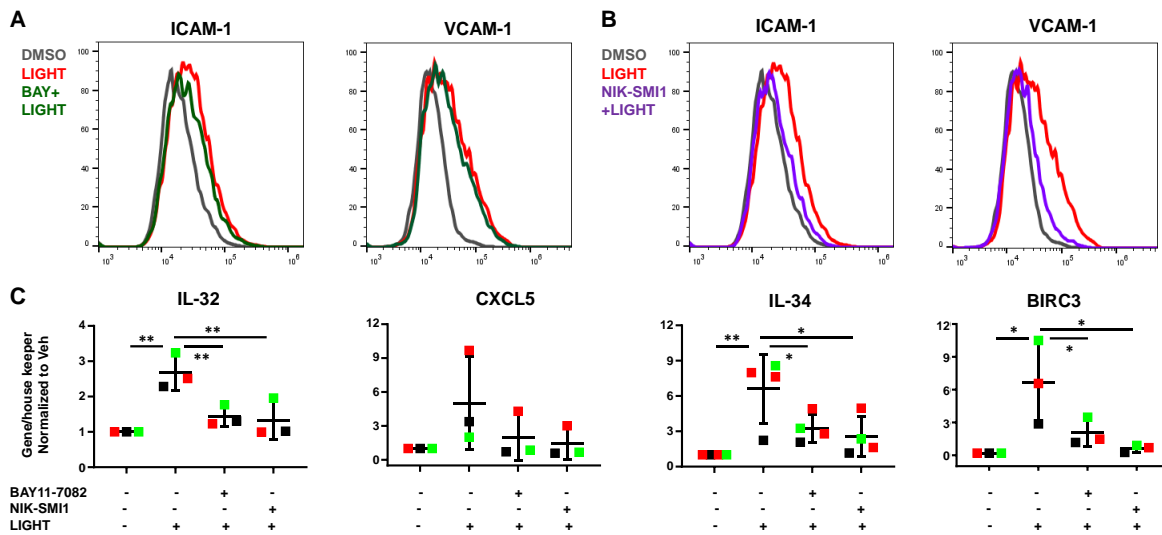
Signaling pathways



Supplementary figure 2



Supplementary figure 3



Supplementary Figure 1. Ingenuity Pathway Analysis of LIGHT down-regulated genes showing relevant diseases and functions and signaling pathways (n=4, >1.5 fold, p<0.05).

Supplementary figure 2. A, quantification of % of HVEM and LT β R positive cells normalized to isotype from plots shown in Figure 1C (n=6, *p<0.05). Validation of HVEM and LT β R silencing in cells transfected with scramble RNA (siNTC) or siRNAs against HVEM (siHVEM) or LT β R (siLT β R) and untreated or treated with LIGHT for 24 hours analyzed by RT-PCR (B, n=4), or in cells transfected with siNTC and siHVEM or siLT β R (C, n=3) or with pCMV6-XL4 or pCMV6-XL4L-HVEM analyzed by flow cytometry (D, n=3). Each colored dot represents fibroblasts from an independent donor. *p<0.05 and ***p<0.001.

Supplementary figure 3. Representative histograms of flow cytometry of ICAM-1 and VCAM-1 in normal esophageal fibroblasts pre-treated with BAY11-7082 (A) or NIK-SMI1 (B) for 1 h and then treated with LIGHT for 4h (n=3). RT-PCR of inflammatory genes in esophageal fibroblasts pre-treated with BAY11-7082 or NIK-SMI1 for 1h and then treated with LIGHT for 4h (C, n \geq 3). Each color dot represents fibroblasts from an independent donor. *p<0.05, **p<0.01.