VEGFR2 trafficking by KIF13B is a novel therapeutic target for wet AMD

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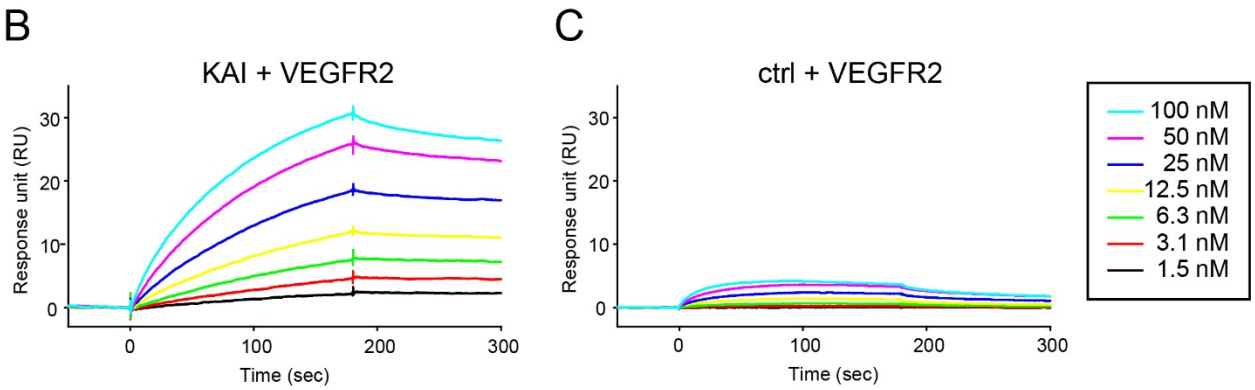
Supplementary information

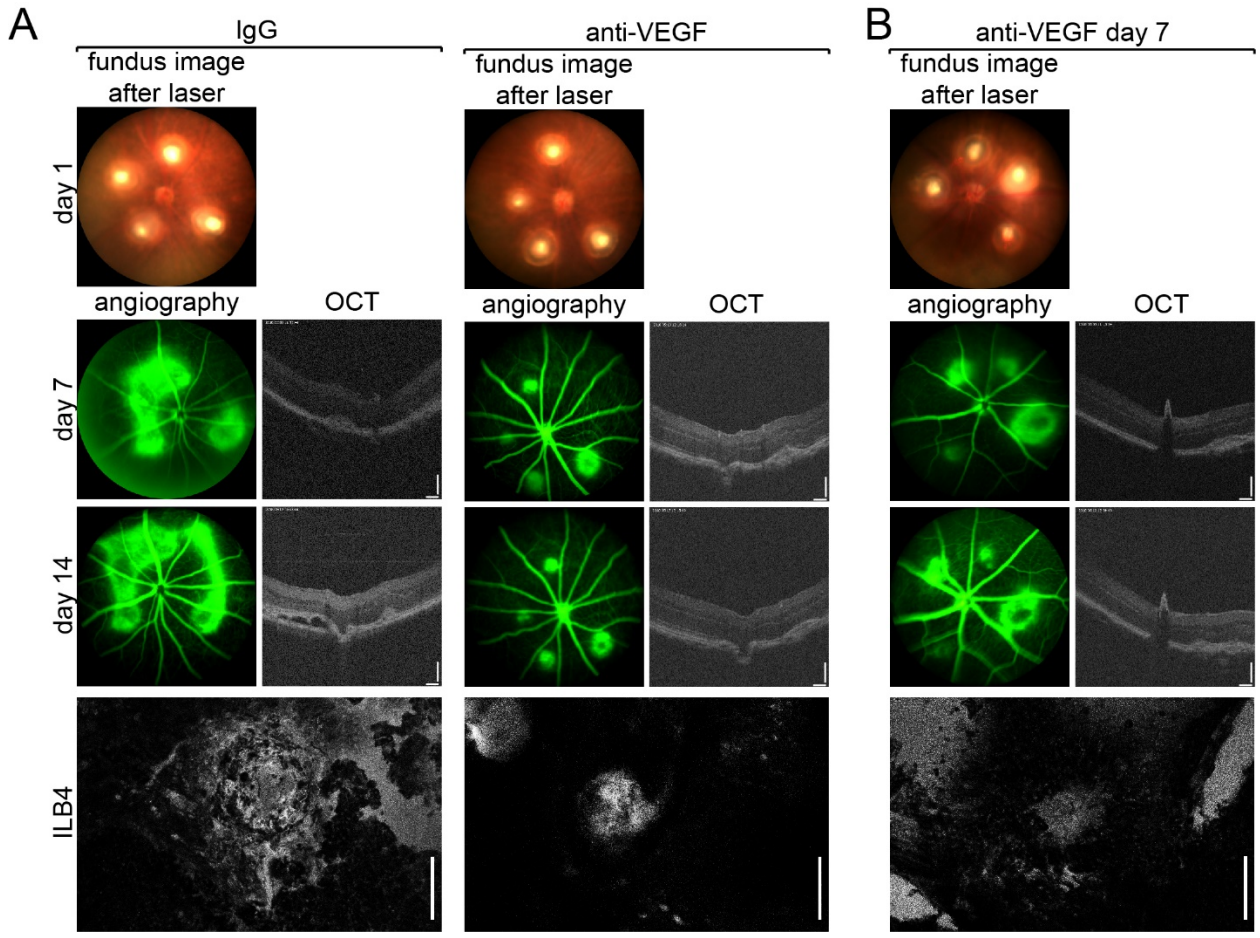
Supplementary Figures and Figure Legends

A

Unique	Total	Gene	XCorr	ΔCorr
24	28	KIF13B	5.477	0.478
11	11	VEGFR2	5.079	0.528
5	6	PDGFR a	4.858	0.509
5	5	PDGFR b	4.838	0.501
5	5	NRP1	4.841	0.585
1	1	EGFR		

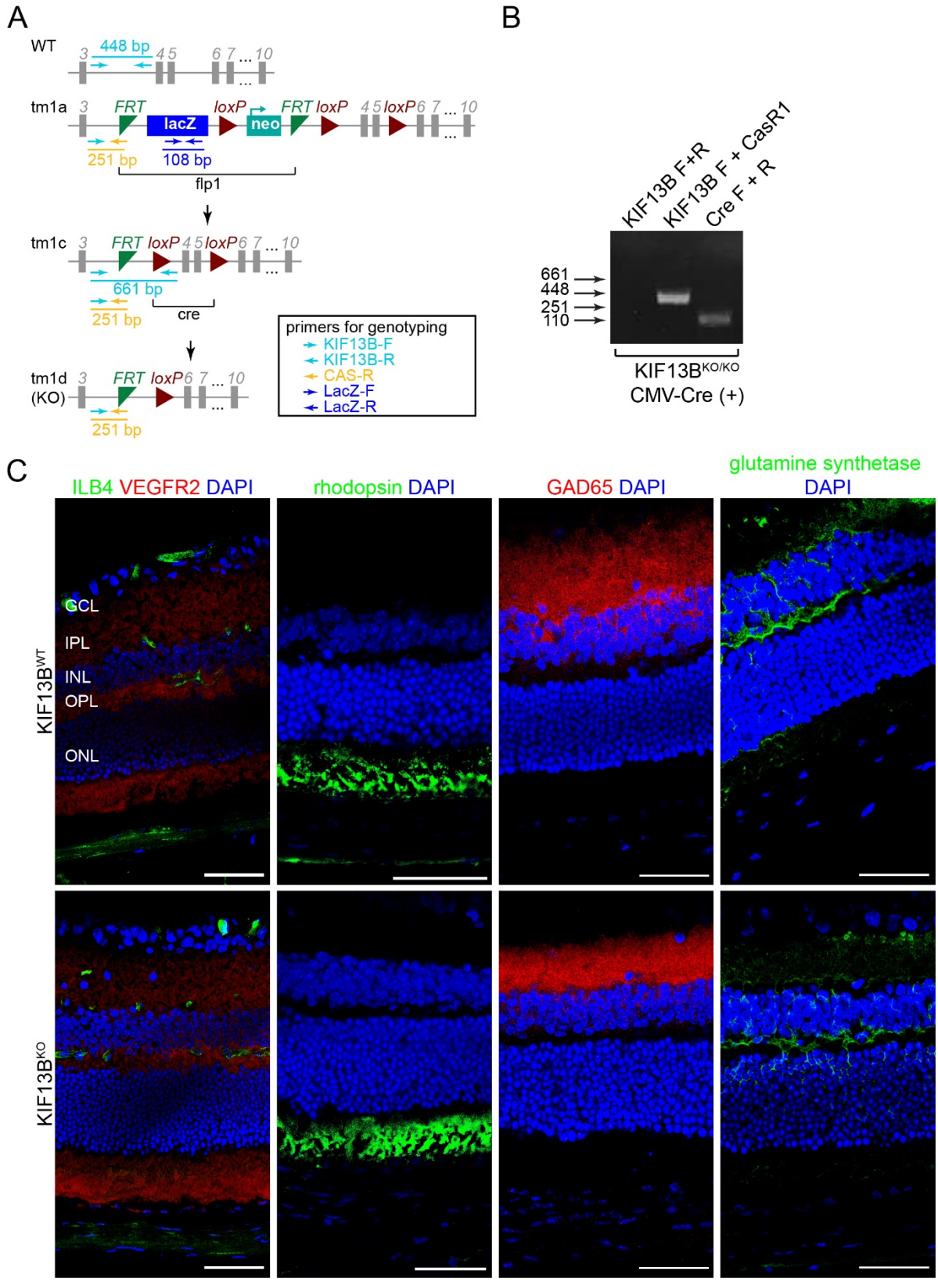


Supplementary Figure 1. VEGFR2 has a high affinity to KAI, not to ctrl (A) Mass spectrometry analysis of the proteins which bound to the biotin-KAI immobilized on the streptavidin beads. After the digestion of the proteins, peptide fragments were analyzed based on the molecular weight to determine the parental proteins. The number of the total and unique peptide fragments, the name of the determining gene, cross-correlation (XCorr), and delta correlation (ΔCorr) were shown in the table. (B) Biotin-KAI was immobilized on the streptavidin (SA) sensor chip surface, and affinity to the recombinant protein of cytosolic VEGFR2 was tested. (C) Biotin-ctrl was immobilized on the SA sensor chip. Even a high concentration of VEGFR2 did not show any affinity to ctrl.

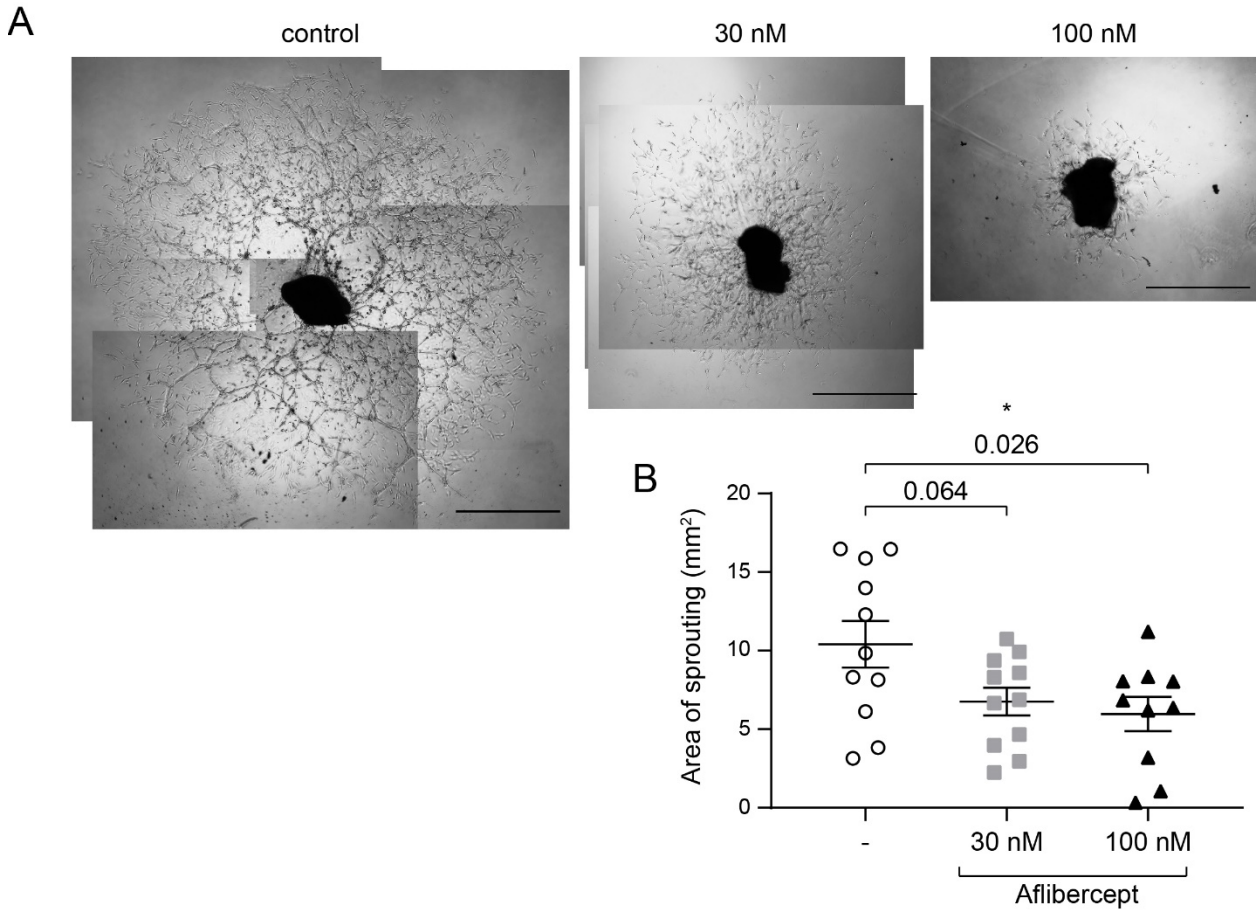


Supplementary Figure 2. Comparison of the efficacy of our strategy with current therapy for wet AMD, anti-VEGF. (A) Efficacy of anti-VEGF antibody on laser-induced CNV. After laser photocoagulation, mice received intravitreal injection of IgG or anti-VEGF antibody 2 μg/eye on day 1. Represent images of the laser burn at day 1, OCT, and angiography at days 7 and 14, and staining of the flat-mount of choroid/sclera with ILB4 on day 14 were shown. Bars: 100 μm for OCT, 200 μm for ILB4 staining. (B) Regression of CNV by treatment of intravitreal injection anti-VEGF antibody after CNV was developed. After laser burn, mice were left untreated for 7 days. On day 7, group #7 mice received an intravitreal injection of anti-VEGF

antibody 2 μg/eye. The representative images of the fundus image after laser burn at day 1, angiography, and OCT at day 7 before the treatment started, and day 14 after the treatment started were shown. The quantitative data from A and B were analyzed and shown in Fig. 3B, C.


A
WT
448 bp
tm1a
FRT
lacZ
loxP
neo
FRT
loxP
251 bp
108 bp
flp1
tm1c
661 bp
cre
251 bp
tm1d
(KO)
251 bp
primers for genotyping
KIF13B-F
KIF13B-R
CAS-R
LacZ-F
LacZ-R
B
KIF13B F+R
KIF13B F + CasR1
Cre F + R
661
448
251
110
KIF13B^KO/KO
CMV-Cre (+)
C
ILB4 VEGFR2 DAPI
rhodopsin DAPI
GAD65 DAPI
glutamine synthetase
DAPI
GCL
IPL
INL
OPL
ONL
KIF13B^WT
KIF13B^KO

Supplementary Figure 3. Generation of global KIF13B KO mice (A) Scheme of KIF13B WT and a mutant allele. (B) Representative image of genotyping shows that the WT allele using *KIF13B-F* and *KIF13B-R* primers (448 bp) was absent in $KIF13B^{KO}$. The existence of FRT cassettes was confirmed with *KIF13B-F* and *CAS-R* primers (251 bp). CMV-Cre was also confirmed with Cre-F and Cre-R primers (100 bp). (C) Immunostaining of the cryosection of the eyes from $KIF13B^{WT}$ and $KIF13B^{KO}$. Intact eyes were isolated from $KIF13B^{WT}$ and $KIF13B^{KO}$. Cryosection of the eyes was analyzed by immunostaining with VEGFR2, ILB4, rhodopsin, GAD65, and glutamine synthetase. No abnormality was noted in $KIF13B^{KO}$. Scale bar: 50 μm.



Supplementary Figure 4. VEGF-dependent sprouting from choroidal tissue *ex vivo*. (A, B) Choroidal tissue fragments were isolated from C57BL/6 mouse eyes, embedded in Matrigel, and incubated in basal media supplemented with 2% FBS without growth factors with or without Aflibercept 30 nM or 100 nM. Tissue fragments dissected from the same animal were divided into 3 groups (control, 30 nM, and 100 nM Aflibercept) and tested at the same time. The independent experiment was repeated 3 times. The sprouting area from each fragment was measured and plotted in graph B as average ± SE. N=11, 11, 10, for control, 30, 100 nM Aflibercept, respectively. N is the number of fragments in each group from 3 independent experiments. One-way ANOVA.