Supplemental materials

Intravitreal anti-VEGF therapy blocks inflammatory cell infiltration and re-entry into the circulation in retinal angiogenesis

Shintaro Nakao¹, Mitsuru Arima¹, Keijiro Ishikawa¹, Riichiro Kohno¹, Shuhei Kawahara¹, Masanori Miyazaki¹, Shigeo Yoshida¹, Hiroshi Enaida¹, Ali Hafezi-Moghadam²,

Toshihiro Kono³, Tatsuro Ishibashi¹
Supplemental Figure 1
Supplemental Figure 2

**A**

IgG  αVEGF Ab

24h

AO

8h

AO

**B**

![Bar graph](http://example.com/bar_graph.png)

\[P = 0.2\]

AO(+) cells/area

IgG  αVEGF Ab

24h

**C**

![Bar graph](http://example.com/bar_graph.png)

\[P = 0.7\]

AO(+) cells/area

IgG  αVEGF Ab

8h
Supplemental Figure 1: AO-stained transmigrated leukocytes in OIR retina. Double staining of retinal flat mounts for transmigrated leukocytes (AO, green) and perfused blood vessels (ConA, red) in a flat-mounted OIR retina (P17). Bar, 200µm.

Supplemental Figure 2: Effect of anti-VEGF Ab on leukocyte infiltration in peripheral non-angiogenic vessels. IgG or αVEGF Ab was treated at P16. After 8 or 24 hours of Ab injection, AO was injected and 2 hours AO(+) leukocyte was examined. A) Representative images of AO(+) cells (arrows) in a flat-mounted OIR retina with IgG or αVEGF Ab treatment. Bar, 200µm. B, C) Quantititation of the number of AO(+) cells in retina 24 (B) or 8 hours (C) after Ab treatment (n=4-7).

Supplemental Figure 3: VEGF-A expression in transmigrated leukocytes in retinal angiogenesis. Representative images of lectin (green), CD45 (red) and VEGF-A (blue) immunostaining in a flat-mounted OIR retina (P17). Bar, 200µm.