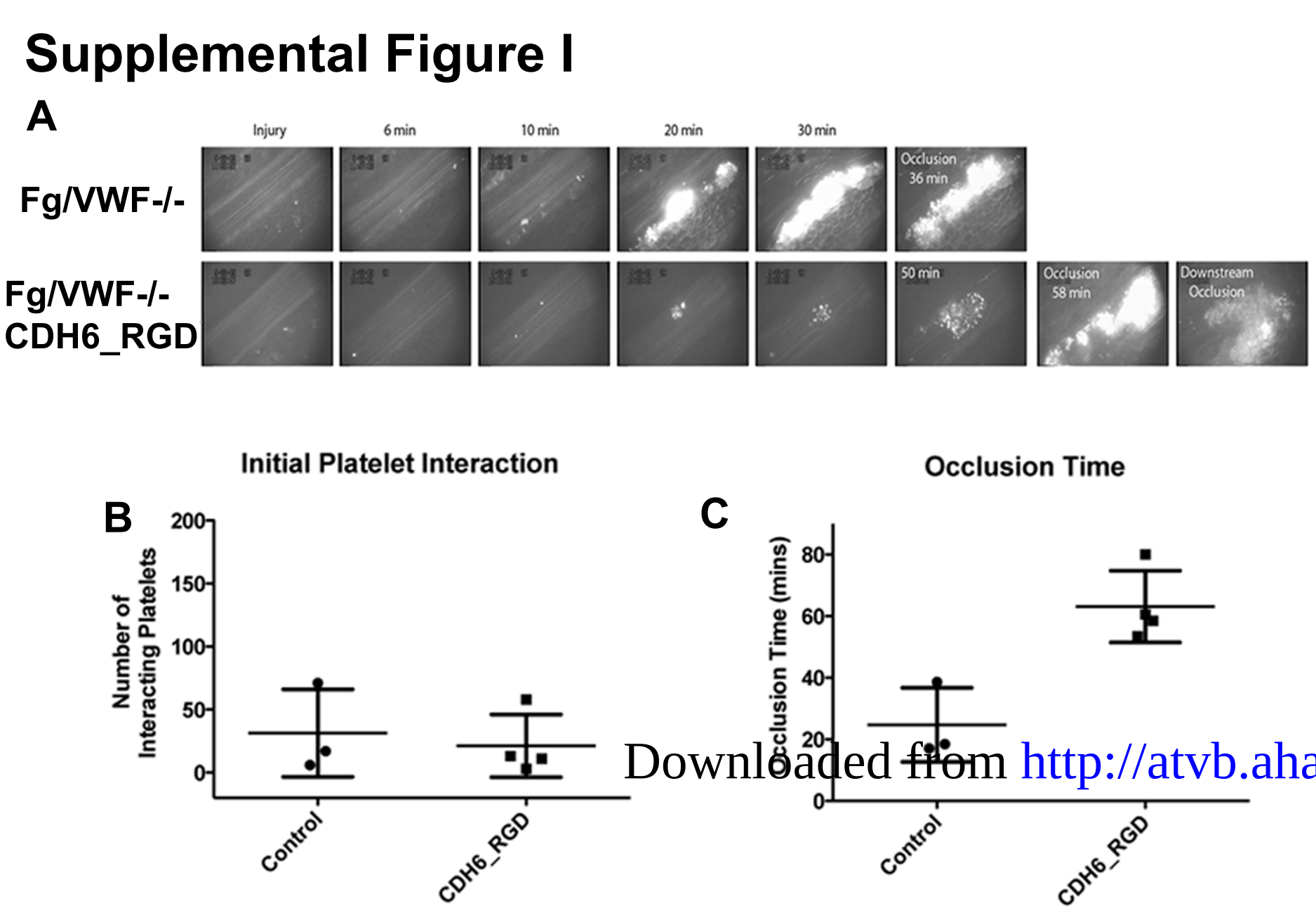
Supplemental Figure I



Supplement Material:

Methods

Intravital microscopy thrombosis models

Mesenteric model: Thrombus formation in arterioles was monitored in 3-4 week old Fg/VWF-/- mice injected with donor-matched fluorescently-labeled platelets under a Zeiss Axiovert 135-inverted fluorescent microscope as previously described [1, 13, 14]. Injury was induced by topical application of 30 μL of 250 mM $FeCl_3$ and thrombus formation was compared between groups based on: (1) number of fluorescent platelets deposited on the vessel wall during the first 3-5 minutes following injury, and (2) time to complete vessel occlusion.

Results

Blockade of Cadherin 6 inhibits thrombus formation in the absence of Fibrinogen and VWF in vivo

Aggregometry experiments indicated that blockade of Cadherin 6 could largely eliminate platelet aggregation in the absence of fibrinogen and VWF. We therefore wished to determine the role of Cadherin 6 in *in vivo* thrombus formation. Fg/VWF-/- double-deficient mice were injected with CDH6_RGD and thrombus formation was induced by $FeCl_3$ as we previously described[1, 14, 16]. In both peptide-treated and untreated Fg/VWF-/- double-deficient mice,

there was no significant difference between the number of platelets initially interacting with the vessel wall, indicating reproducible injury of comparable magnitude between the two groups of mice (Fig 5, $p = 0.671$). The mean vessel occlusion time was however significantly delayed in the mice treated with the CDH6_RGD peptide, with one peptide-treated mouse showing no vessel occlusion after 1 hour and 20 minutes of continuous monitoring (Fig 5, $p = 0.008$).

Vessel occlusion in the peptide-treated mice also differed qualitatively from the untreated mice. In Peptide-treated mice thrombi tended to be more discreet and spatially restricted as opposed to the thrombi in untreated mice which were prolific and disseminated along the length of the damaged arteriole. Furthermore, thrombi in peptide treated mice were unstable and only grew to an occlusive size once downstream embolization slowed the arterial flow.

Figure I: Blockade of Cadherin 6 results in decreased thrombus formation and vessel occlusion

A: Mesenteric arterioles from Fg/VWF-/- mice were injured with $FeCl_3$ and the number of platelets interacting with the damaged vessel wall was monitored in the first 3-5 min. Vessel occlusion time was also recorded. B: The number of initially interacting platelets remained comparable between groups ($p = 0.671$). C: However, mice treated with Cadherin 6 peptide (CDH6_RGD) corresponding to a portion of the extracellular domain containing the RGD sequence demonstrated a significant delay in occlusive thrombus formation ($p = 0.008$). In CDH6_RGD peptide treated mice, vessel occlusion was

dramatically delayed and the thrombi were less dispersed around the area of injury. These mice also tended to form downstream occlusions, rather than occlusions at the site of vessel injury