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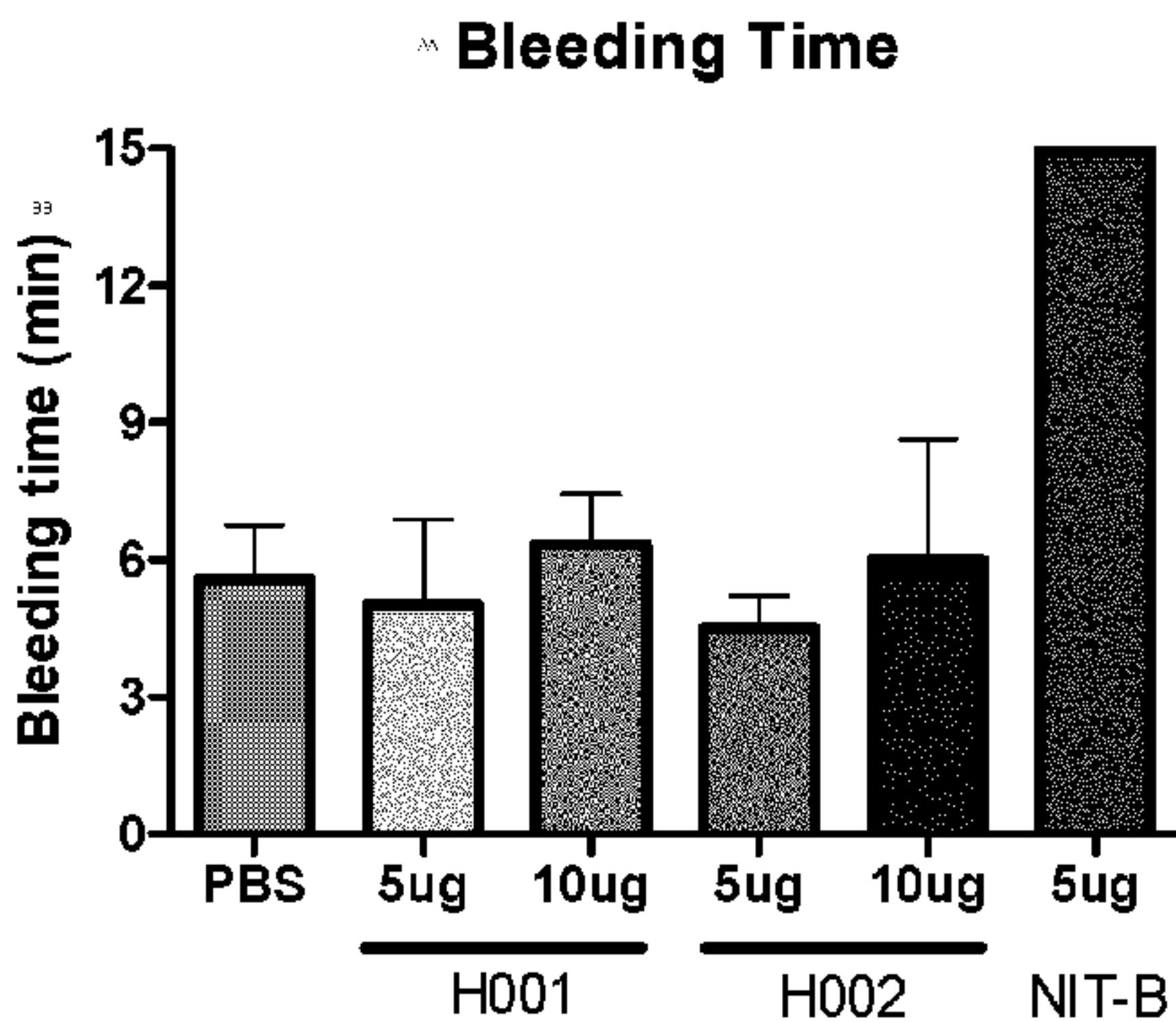
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(54) Title: HUMANIZED ANTI-GLYCOPROTEIN IB ALPHA (GPIBALPHA) ANTIBODIES

Figure 18



(57) Abstract: Multivalent anti-platelet glycoprotein I(b)alpha antibodies can cause severe side effects. The present disclosure provides humanized antibodies specifically recognizing glycoprotein I(b)alpha and lacking a Fc portion, therefore do not interact with Fc receptor. The humanized antibodies are capable of preventing platelet activation and aggregation, and reducing thrombus size / growth and prevent vessel occlusion. They can be also very useful to decrease platelet-tumor cell interaction and decrease tumor metastasis. At therapeutic doses, the humanized antibodies lack the ability to induce platelet activation, induce thrombocytopenia; and/or prolong bleeding time.

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HUMANIZED ANTI-GLYCOPROTEIN IB ALPHA (GPIBALPHA) ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS AND SEQUENCE LISTING STATEMENT

5 This application claims priority from U.S. provisional application serial number 62/946,086
filed on December 10, 2019 and incorporated herewith in its entirety. The sequence listing
associated with this application is provided in text format and is hereby incorporated by
reference into the specification. The name of the text file containing the sequence listing is
PCT_-_Sequence_listing_as_filed. The text file is 81.2 Ko, was created on December 9,
10 2020 and is being submitted electronically.

TECHNOLOGICAL FIELD

The present disclosure concerns humanized antibodies which specifically recognize and bind
to the platelet glycoprotein I(b) α (GPIb α) as well as protein construct comprising same, and
therapeutic uses associated thereto.

15 BACKGROUND

Platelet adhesion and aggregation at sites of atherosclerotic rupture in coronary or cerebral
arteries are usually the critical events in acute thrombosis. Therefore, anti-platelet therapy
represents one of the key treatment regimens in reducing cardiovascular deaths, including (i)
cyclooxygenase inhibitors such as aspirin; (ii) platelet P2Y₁₂ receptor antagonists such as
20 clopidogrel, prasugrel and ticagrelor; (iii) α IIb β 3 antagonists such as abciximab, eptifibatide
and tirofiban; and (iv) PAR1 antagonists such as vorapaxar, etc. However, limitations of
current anti-platelet therapies, such as a slow onset/weak/poor inhibition of platelet function,
excessive bleeding complications, thrombocytopenia and unexpected platelet activation are
major concerns that drive therapeutic advances. Notably, concerning acute ischemic stroke,
25 since the risk for intracranial hemorrhage and/or potential neurotoxicity (e.g. recombinant
tissue plasminogen activator (tPA)) of current anti-thrombotic/thrombolytic drugs, as well as
for patients who have passed the window for intravenous thrombolysis, treatments are very
limited if it is not available.

Platelet GPIb-IX-V complex has emerged as a promising anti-platelet target. GPIb-IX-V
30 complex is a key platelet receptor in initiating platelet adhesion and translocation to the
injured vessel wall, particularly at high shear. Platelet adhesion/translocation onto the
subendothelium is mediated by the binding of GPIb α subunit to von Willebrand Factor (VWF)
that anchored/immobilized on the injured vessel wall. VWF is a multimeric adhesive blood
protein secreted from activated endothelial cells and platelets. GPIb α -VWF binding can then

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trigger a signal transduction process that leads to the release of platelet agonists, such as thromboxane A₂ and ADP, and the activation of platelet α IIb β 3 integrin that results in platelet aggregation mediated by α IIb β 3 binding to fibrinogen, VWF, etc. Under high shear conditions, the GPIb α -VWF interaction are required for pathologic growth of occlusive thrombi (both platelet adhesion and platelet aggregation/agglutination) at sites of arterial stenosis where blood flows with wall shear rates that may exceed 10 000-40 000 s⁻¹, while under low shear conditions (such as in the most cases of hemostasis), platelet adhesion can be directly mediated by α IIb β 3-fibrinogen/fibrin and α 2 β 1/GPVI-collagen interactions, etc. Therefore, pharmacological inhibition of GPIb α may result in a lower risk of systemic bleeding and improved safety compared to other antiplatelet drugs that are not specifically target thrombosis at high shear. It has also been shown that GPIb α is important for leukocyte recruitment under thrombo-inflammatory conditions, such as in acute ischemic stroke. Moreover, ischemia – reperfusion (e.g. by thrombolysis or thrombectomy) of the previously hypoxic brain areas can increase the pro-inflammatory function of platelets via GPIb α , which may further promote thrombo-inflammatory neuronal damage and infarct growth. Furthermore, GPIb α has been considered exclusively expressed on platelets and megakaryocytes. Thus, direct platelet GPIb α antagonists have a great potential to be developed as effective and safer anti-platelet drugs for the treatment of acute thrombotic events, such as heart attack and stroke.

Notably, novel anti-platelet strategies targeting GPIb α -VWF interaction has been demonstrated as an effective therapy to treat acquired thrombotic thrombocytopenic purpura (aTTP), a thrombotic microangiopathy and a life-threatening condition with a high mortality rate if untreated. Autoantibodies against ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13), the VWF-cleaving protease that cleaves/reduces the multimeric size of VWF, results in the severe deficiency of ADAMTS13 activity. These ultra-large VWF are hyper-adhesive that cause the formation of platelet (GPIb α)-VWF microthrombi in blood vessels, leading to the end-organ ischemia and infarction, low platelet counts and destruction of red blood cells. Therefore, blocking the interaction between VWF and platelet GPIb α can prevent the development of acute TTP by achieving faster normalization of platelet counts and reducing the thromboembolic events.

The nanobody caplacizumab targeting the VWF A1 domain has been approved for the treatment of adults experiencing an acute episode of aTTP, in conjunction with plasma exchange (PEX) and immunosuppression for a minimum of 30 days after stopping daily PEX. However, bleeding-related adverse events were more common with caplacizumab (65% vs. 48%), as were serious bleeding events (11% vs. 1%). Another barrier of caplacizumab has been the huge cost of the drug. Current pricing of caplacizumab in 2020 was more than

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US\$8,000 per dose with treatment regimens recommending daily dosing for 30 days following the last plasma exchange with the possibility of extended treatment pending the recovery of ADAMTS13 activity. Due to that VWF is consistently released from the activated endothelium, the relative stable levels of GPIb α on platelets, which have a short lifespan of 5 7- 10 days in humans, appears to be a more attractive/potent target for TTP therapy.

Patent CN103263662 describes a GPIb α -binding snake C-type lectin (snaclec) anti-platelet thrombolytic which is purified from the venom of the snake *Deinagkistrodon acutus*. Anti-platelet thrombolytic inhibited ristocetin-induced human platelet aggregation and thrombosis through blocking GPIb-VWF interactions without significantly altering bleeding time or 10 coagulation. Anti-platelet thrombolytic is currently evaluated in the phase II clinical trials in patients with acquired thrombotic thrombocytopenic purpura and ST segment elevation myocardial infarction. However, it is known that foreign snake proteins may induce an immune response that generates anti-drug antibodies which can neutralized the drug and eliminate the therapeutic efficacy. Further, these antibodies may cause allergic reactions or 15 immune complex formation that may damage kidney or joints (arthritis). Furthermore, the need for re-administration may stimulate the memory immune cells and boost such immune responses and immune reactions.

US Patent Serial Number 7,049,128 describes recombinant GPG-290 or GPIb-290/2V-Immunoglobulin (Ig) fusion polypeptides, which is a soluble chimeric protein containing the 20 mutant GPIb α N-terminal extracellular 290 amino acids (G233V and M239V) linked via a proline to the Fc fragment of human IgG1. GPG-290 competed with platelet GPIb α for binding VWF, with a fourteen-fold affinity for VWF compared with the wild-type GPIb α . In animal models, GPG-290 dose-dependently prolonged the time to coronary artery occlusion, and inhibited platelet aggregation, thrombosis and recurrent coronary cyclic flow reduction 25 without prolonging the bleeding time at doses ranging from 50-100 μ g/kg. However, the higher dose tested (500 μ g/kg) induced three to fourfold increase of the bleeding time, likely due to that GPG-290 binding to alpha-thrombin with an high affinity which makes the alpha-thrombin is not available for hemostasis.

US Patent Serial Number 7,727,535 further describes a GPIb-290/2V/FFF-Ig variant fusion 30 protein (Y276F, Y278F, and/or Y279F), which exhibited a limited/lower affinity binding to alpha-thrombin, and had a 50% decrease in potency in inhibiting repetitive coronary artery thrombosis (i.e. inhibiting recurrent coronary cyclic flow reduction), and in prolonging tail bleeding times and increasing ADP closure times as assessed by a Platelet Function Analyzer-100 (PFA-100) as compared to GPG-290. However, these GPIb-Ig fusion proteins 35 are high molecular weight chimeric protein (~130 kDa), which mainly targets the subendothelium immobilized VWF or plasma VWF under high shear rates. Therefore, the

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amount and accessibility of these fusion proteins are less well predictable and the high amount of the products to be injected could be a potential limitation. Another limitation could be the risk of anti-drug antibody generation. The GPIb-Ig fusion proteins are bioengineered chimera proteins. Although both GPIb α polypeptides and Fc fragment are derived from
5 human genes, which may minimize their antigenicity, the GPIb α variant mutations and the joint regions between GPIb α and Fc portion may generate neoepitopes that may induce an immune response. In addition, the fusion protein may generate some conformational neoepitopes that induce anti-drug antibody production.

Neutralizing monoclonal antibodies directed against human GPIb α (anti-GPIb α mAbs) were
10 also described in the art. However, the majority anti-GPIb α mAbs available to-date are of murine origin, and therefore can elicit a human anti-mouse response in clinical use. In addition, the intact anti-GPIb α mAbs often lead to platelet activation, likely due to that the binding of intact mAbs induced platelet GPIb α -mediated signaling transduction, which can unexpectedly aggravate platelet aggregation and thrombosis. Furthermore, the intact anti-
15 GPIb α mAbs binding to platelets can trigger both Fc-dependent and Fc-independent platelet clearance, leading to thrombocytopenia (i.e. low platelet counts). Therefore, the intact anti-GPIb α mAbs present a very limited therapeutic potential.

CN102988983 describes a Fab fragment of chimeric antibody chSZ2, a chimeric mAb against human GPIb α , that inhibits ristocetin-induced platelet aggregation *in vitro* in a dose-
20 dependent manner. However, since it is a chimeric antibody which retains the variable regions of mouse antibody and the constant regions are replaced by those of human, the immunogenicity is still a major concern. Importantly, the *in vivo* function of chSZ2 for preventing/treating thrombotic diseases have never been demonstrated, due to shortage of animal models since it may not recognize GPIb α from other animal species as well
25 recognized in the field.

US Patent Serial Number 7,332,162 describes another Fab fragment of the humanized 6B4 (h6B4-Fab), a murine mAb which was raised against purified human GPIb α . h6B4-Fab reduced or completely abolished cyclic flow reductions of a stenosed femoral artery in baboons. However, the antithrombotic effect of h6B4-Fab was accompanied by a
30 prolongation of the bleeding time. Moreover, these anti-GPIb α antibodies, as well as their corresponding Fab fragments, were generated in wild-type mice using conventional techniques (i.e. immunized by human GPIb α) which cannot recognize the mouse GPIb α (because mouse and human GPIb α possess an important degree of homology), and the repertoire of the antibodies produced to epitopes present on the human GPIb α and absent
35 on the mouse GPIb α is limited. Therefore, these mAbs cannot be characterized and evaluated in rodents or other animal species for the important preclinical pharmacology,

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toxicology and pharmacokinetics studies. Whether h6B4-Fab may cause platelet activation is also of concern since its precursor mAb 6B4 can clearly cause platelet activation and severe thrombocytopenia.

There is thus a need for an improved therapeutic agent targeting the platelet GPIb-IX-V complex which would not cause platelet activation, platelet destruction, thrombocytopenia, nor significant bleeding complications.

BRIEF SUMMARY

The present disclosure concern a humanized and antibody specifically recognizing glycoprotein I(b) α (GPIb α) and protein construct comprising same as well as therapeutic uses associated thereto. The humanized antibody is capable of preventing platelet activation, aggregation, thrombus growth (particularly at high shear), lacks the ability to activate platelets (e.g., does not activate platelets), lacks the ability to induce thrombocytopenia; and/or at a therapeutic dose, lacks the ability to prolong bleeding time.

In a first aspect, the present disclosure provides a humanized antibody specifically recognizing glycoprotein I(b) α (GPIb α). The humanized antibody lacks a Fc receptor moiety. The humanized antibody is capable of preventing platelet activation, aggregation, and/or thrombus growth; lacks the ability to activate platelets; lacks the ability to induce thrombocytopenia; and/or at a therapeutic dose, lacks the ability to prolong bleeding time. In an embodiment, the humanized antibody is capable of recognizing a human GPIb α , a mouse GPIb α , a dog GPIb α , a rat GPIb α , a rabbit GPIb α and/or a monkey GPIb α . In another embodiment, the humanized antibody is an antibody fragment. In an example, the antibody can be a F(ab)₂ fragment. For example, the antibody fragment can be a Fab antibody fragment. In another embodiment, the antibody fragment can be a single chain variable fragment (scFv). In an embodiment, the humanized antibody of has a heavy chain. In some embodiments, the heavy chain comprises: a first CDR having an amino acid sequence of GFTFSSFAMS (SEQ ID NO: 37), a variant thereof or a fragment thereof; a second CDR having an amino acid sequence of SITSAGTPYYPDSVLG (SEQ ID NO: 38), a variant thereof or a fragment thereof; and/or a third CDR having an amino acid sequence of SRGYEDYFDY (SEQ ID NO: 39), a variant thereof or a fragment thereof. In still a further embodiment, the heavy chain further comprises a CH1 region of a human IgG₁ antibody. For example, the CH1 region of the human IgG₁ antibody has the amino acid sequence of SEQ ID NO: 40, 47, 54 or 61, a variant thereof or a fragment thereof. In an embodiment, the heavy chain has the amino acid sequence of SEQ ID NO: 36, 43, 50 or 57, a variant thereof or a fragment thereof. In another embodiment, the humanized and monoclonal antibody has a light chain. In some embodiments, the light chain comprises: a first CDR having an amino

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acid sequence of KSSQSLLNSRNQKNYLA (SEQ ID NO: 65), a variant thereof or a fragment thereof; a second CDR having an amino acid sequence of FTSTRES (SEQ ID NO: 66), a variant thereof or a fragment thereof; and/or a third CDR having an amino acid sequence of QQHYSSPWT (SEQ ID NO: 67), a variant thereof or a fragment thereof. In some
5 embodiments, the light chain further comprises a kappa chain C region of a human IgG₁ antibody. In some additional embodiments, the kappa chain C region has the amino acid sequence of SEQ ID NO: 68, 75, 82, or 89, a variant thereof or a fragment thereof. In a further embodiment, the light chain has the amino acid sequence of SEQ ID NO: 64, 71, 78 or 85, a variant thereof or a fragment thereof. In some embodiments, the humanized
10 antibody has the heavy chain of SEQ ID NO: 36, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 64, a variant thereof or a fragment thereof; the heavy chain of SEQ ID NO: 36, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 71, a variant thereof or a fragment thereof; the heavy chain of SEQ ID NO: 36, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 78, a variant thereof or a fragment
15 thereof; the heavy chain of SEQ ID NO: 36, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 85, a variant thereof or a fragment thereof; the heavy chain of SEQ ID NO: 43, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 64, a variant thereof or a fragment thereof; the heavy chain of SEQ ID NO: 43, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 71, a variant thereof or a fragment
20 thereof; the heavy chain of SEQ ID NO: 43, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 78, a variant thereof or a fragment thereof; the heavy chain of SEQ ID NO: 43, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 85, a variant thereof or a fragment thereof; the heavy chain of SEQ ID NO: 50, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 64, a variant thereof or a fragment
25 thereof; the heavy chain of SEQ ID NO: 50, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 71, a variant thereof or a fragment thereof; the heavy chain of SEQ ID NO: 50, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 78, a variant thereof or a fragment thereof; the heavy chain of SEQ ID NO: 50, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 85, a variant thereof or a fragment
30 thereof; the heavy chain of SEQ ID NO: 57, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 64, a variant thereof or a fragment thereof; the heavy chain of SEQ ID NO: 57, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 71, a variant thereof or a fragment thereof; the heavy chain of SEQ ID NO: 57, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 78, a variant thereof or a fragment
35 thereof; or the heavy chain of SEQ ID NO: 57, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 85, a variant thereof or a fragment thereof.

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In a second aspect, the present disclosure provides a chimeric protein comprising the humanized antibody of described herein and a carrier protein.

In a third aspect, the present disclosure provides a pharmaceutical composition comprising (i) the humanized antibody described or the chimeric protein described herein and (ii) a
5 pharmaceutical excipient.

In a fourth aspect, the present disclosure provides a method of preventing or limiting the interaction between glycoprotein I(b) α (GPIb α) present on a platelet and a GPIb α ligand (such as, for example, von Willebrand factor (VWF), thrombin, kininogen, P-selectin, thrombospondin, etc.), the method comprising contacting the humanized antibody described
10 herein, the chimeric protein described herein or the pharmaceutical composition described herein with the platelet. In some embodiments, the method is for preventing or limiting platelet activation. In an embodiment, the GPIb α ligand is the von Willebrand factor (VWF) and/or thrombin. In a specific embodiment, the humanized antibody, the chimeric protein or the pharmaceutical composition is contacted with the platelet prior to, at the same time or
15 after the GPIb α ligand is contacted with the platelet. In a further embodiment, the method is for preventing or limiting the interaction *in vivo* in a subject in need thereof. In another embodiment, the method is for preventing the formation or the growth of a thrombus in the subject in need thereof. In another embodiment, the method is for reducing the size of a thrombus or the number of thrombi in the subject in need thereof. In some embodiments, the
20 method further comprises determining the presence, the location and/or the size of the thrombus in the subject. In yet another embodiment, the subject is at risk of experiencing or has experienced a pathological thrombosis. In yet another embodiment, the subject is at risk of experiencing or has experienced an ischemic stroke, a thrombotic thrombocytopenic purpura, a myocardial infarct, an acute coronary syndrome, atherothrombosis, a peripheral
25 vascular disease, deep vein thrombosis, sepsis, and/or vascular inflammation. In some embodiments, the method is for reducing or limiting tumor metastasis in the subject in need thereof. In further embodiment, the tumor metastasis are liver tumor metastasis. In yet another embodiment, the method further comprises determining the presence, the location and/or the size of the tumor metastasis in the subject.

30 In a fifth aspect, the present disclosure provides using the humanized antibody described herein, the chimeric protein described herein or the pharmaceutical composition for preventing or limiting the interaction between glycoprotein I(b) α (GPIb α) present on a platelet and von Willebrand factor (VWF) and/or thrombin as well as other GPIb α ligands. The present disclosure also provides using the humanized antibody described herein, the
35 chimeric protein described herein or the pharmaceutical composition in the manufacture of a

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medicament for preventing or limiting the interaction between glycoprotein I(b) α (GPIb α) present on a platelet and von Willebrand factor (VWF) and/or thrombin. The contacting step can occur under low or high shear rates. In some embodiments, the humanized and monoclonal antibody, the chimeric protein or the pharmaceutical composition is for preventing or limiting platelet activation. In a specific embodiment, the humanized antibody, the chimeric protein or the pharmaceutical composition is for contacting with the platelet prior to, at the same time or after the VWF and/or thrombin as well as other GPIb α ligands is contacted with the platelet. In a further embodiment, the humanized and monoclonal antibody, the chimeric protein or the pharmaceutical composition is for preventing or limiting the interaction *in vivo* in a subject in need thereof. In another embodiment, the humanized and monoclonal antibody, the chimeric protein or the pharmaceutical composition is for preventing the formation or the growth of a thrombus in the subject in need thereof. In another embodiment, the humanized and monoclonal antibody, the chimeric protein or the pharmaceutical composition is for reducing the size of a thrombus or the number of thrombi in the subject in need thereof. In some embodiments, the presence, the location and/or the size of the thrombus was previously determined in the subject. In yet another embodiment, the subject is at risk of experiencing or has experienced a pathological thrombosis. In yet another embodiment, the subject is at risk of experiencing or has experienced an ischemic stroke, a thrombotic thrombocytopenic purpura, a myocardial infarct, an acute coronary syndrome, atherothrombosis, a peripheral vascular disease, deep vein thrombosis, sepsis, and/or vascular inflammation. In some embodiments, the humanized and monoclonal antibody, the chimeric protein or the pharmaceutical composition is for reducing or limiting tumor metastasis in the subject in need thereof. In further embodiment, the tumor metastasis are liver tumor metastasis. In yet another embodiment, the presence, the location and/or the size of the tumor metastasis have previously been determined in the subject.

In a sixth aspect, the present disclosure provides a humanized antibody described herein, a chimeric protein described herein or a pharmaceutical composition for preventing or limiting the interaction between glycoprotein I(b) α (GPIb α) present on a platelet and von Willebrand factor (VWF) and/or thrombin. In some embodiments, the humanized and monoclonal antibody, the chimeric protein or the pharmaceutical composition is for preventing or limiting platelet activation. In a specific embodiment, the humanized antibody, the chimeric protein or the pharmaceutical composition is for contacting with the platelet prior to, at the same time or after the VWF and/or thrombin as well as other GPIb α ligands is contacted with the platelet. In a specific embodiment, the humanized antibody, the chimeric protein or the pharmaceutical composition is for contacting with the platelet at a low or a high shear rate. In a further embodiment, the humanized and monoclonal antibody, the chimeric protein or the

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pharmaceutical composition is for preventing or limiting the interaction *in vivo* in a subject in need thereof. In another embodiment, the humanized and monoclonal antibody, the chimeric protein or the pharmaceutical composition is for preventing the formation or the growth of a thrombus in the subject in need thereof. In another embodiment, the humanized and monoclonal antibody, the chimeric protein or the pharmaceutical composition is for reducing the size of a thrombus or the number of thrombi in the subject in need thereof. In some embodiments, the presence, the location and/or the size of the thrombus was previously determined in the subject. In yet another embodiment, the subject is at risk of experiencing or has experienced a pathological thrombosis. In yet another embodiment, the subject is at risk of experiencing or has experienced an ischemic stroke, a thrombotic thrombocytopenic purpura, a myocardial infarct, an acute coronary syndrome, atherothrombosis, a peripheral vascular disease, deep vein thrombosis, sepsis, and/or vascular inflammation. In some embodiments, the humanized and monoclonal antibody, the chimeric protein or the pharmaceutical composition is for reducing or limiting tumor metastasis in the subject in need thereof. In further embodiment, the tumor metastasis are liver tumor metastasis. In yet another embodiment, the presence, the location and/or the size of the tumor metastasis have previously been determined in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the nature of the invention, reference will now be made to the accompanying drawings, showing by way of illustration, a preferred embodiment thereof, and in which:

Figure 1 shows the results of a SDS-PAGE of humanized Fabs in supernatants under non-reducing conditions. The different combinations of heavy and light chains are indicated above the gel. The molecular weight ladder (in KDa) is shown on the left. The arrows point to the humanized Fabs. Bovine serum albumin (BSA) was used as a control.

Figure 2 shows the results of a Western blot results of humanized Fabs under non-reducing conditions. About 20 µL of supernatant was loaded in each lane. The molecular weight ladder (in KDa) is shown on the left. Heavy-chain only (HCAb) antibodies were used as a control.

Figures 3A to H show the results of a SDS-PAGE under non-reducing (labelled as "N") and reducing conditions (labelled as "R") for purified Fabs comprising (**Fig. 3A**) the VH1 and VL2 chains, (**Fig. 3B**) the VH1 and VL3 chains, (**Fig. 3C**) the VH2 and VL1 chains, (**Fig. 3D**) the VH3 and VL2 chains, (**Fig. 3E**) the VH3 and VL3 chains, (**Fig. 3F**) the VH4 and VL1 chains, (**Fig. 3G**) the VH4 and VL2 chains and (**Fig. 3H**) the VH4 and VL3 chains.

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Figures 4A and B show that the humanized Fabs H001 to H008 bind to (Fig. 4A) wild-type mouse platelets but not to (Fig. 4B) GPIIb α -/- mouse platelets (5 μ g/mL). The “*” shown on Figure 4B indicated the control signal.

Figures 5A to E show that purified Fabs H001 ($\blacktriangle$) and H002 ($\blacktriangledown$) bind to (Fig. 5A) mouse, (Fig. 5B) dog, (Fig. 5C) human, (Fig. 5D) rat and (Fig. 5E) rabbit platelets. Humanized Fabs binding to platelets was tested *in vitro* by flow cytometry assay.

Figure 6 shows flow cytometry results that purified Fabs H001 and H002 bind to monkey platelets.

Figure 7 shows that purified Fab H001 antibody binds to recombinant GPIIb α in a surface plasmon resonance (SPR) assay. (Fig. 7A) SPR data of 25 μ L injections of 500, 100, 50 and 10 nM H001 fit to the kinetics binding model producing: k_a (on rate) = $2.61 \times 10^7 \text{ s}^{-1}$; k_d (off rate) = $1.1 \times 10^{-1} \text{ s}^{-1}$; and K_d (dissociation or binding constant) = 4.4 nM. (Fig. 7B) Dose response curve of the SPR response of 25 μ L injections of 500, 100, 50 and 10 nM of the purified Fab H001 plotted against ligand concentration. The curve was fit to a one-site ligand binding model producing a fit $R^2 = 0.9929$ and a $K_d = 8.0 \pm 2.1 \text{ nM}$.

Figures 8A to D show standard aggregometry traces indicating that the purified Fabs (Fig. 8A) H001, (Fig. 8B) H002, (Fig. 8C) H005 and (Fig. 8D) H008 did not induce platelet activation in platelet-rich plasma.

Figures 9A to D show standard aggregometry traces indicating that purified Fabs (Fig. 9A and 9D) H001 and (Fig. 9B, 9C and 9D) H002 inhibited platelet aggregation induced by ristocetin (A, B and D) or low dose thrombin (C). Platelets were obtained from healthy volunteers (A to C) or patients with peripheral vascular disease (D).

Figure 10A to D show that the humanized Fabs H001 (Fig. 10A and 10B) and H002 (Fig. 10C and 10D) antibodies inhibited thrombus formation from human whole blood at both low (300 s^{-1} , Fig. 10A and 10C) and high shear (1800 s^{-1} , Fig. 10B and 10D) rate conditions. (Fig. 10A) Representative photographs showing the platelet thrombus formation after heparinized-human whole blood were perfused for 1, 2, and 3 minutes, which were treated with a control PBS buffer (top panels) and the humanized Fab H001 antibody (5 μ g/mL, bottom panels) at low shear (300 s^{-1}) condition. (Fig. 10B) Representative photographs showing the platelet thrombus formation after heparinized-human whole blood were perfused for 1, 2, and 3 minutes, which were treated with a control PBS buffer (top panels) and the humanized Fab H001 antibody (2.5 μ g/mL, middle panels and 5 μ g/mL, bottom panels) at high shear (1800 s^{-1}) condition. (Fig. 10C) Representative photographs showing the platelet thrombus formation after heparinized-human whole blood were perfused for 1, 2, and 3 minutes, which were treated with a control PBS buffer (top panels) and the humanized Fab H002 antibody (5

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µg/mL, bottom panels) at low shear (300 s⁻¹) condition. (**Fig. 10D**) Representative photographs showing the platelet thrombus formation after heparinized-human whole blood were perfused for 1, 2, and 3 minutes, which were treated with a control PBS buffer (top panels) and the humanized Fab H002 antibody (2.5 µg/mL, middle panels and 5 µg/mL, bottom panels) at high shear (1800 s⁻¹) condition.

Figures 11A and B show that the humanized Fabs H001 and H002 antibodies prolonged vessel occlusion time in a FeCl₃-induced mesenteric arteriole thrombosis model *in vivo*. (**Fig. 11A**) Histogram showing the time to occlusion (in minutes) in function of the antibody or the dose used. (**Fig. 11B**) Representative photographs of arterioles treated with a control (top panels), the humanized Fab H001 antibody (5 µg/mouse, middle panels), the humanized Fab H002 antibody (5 µg/mouse, lowest panels) at different time points where indicated after vessel injury induced by FeCl₃. * P<0.05, ** P<0.01.

Figures 12A to C show that the humanized Fabs H001 and H002 antibodies inhibited thrombus formation in a laser-induced cremaster arteriole thrombosis model *in vivo*. (**Fig. 12A**) Histogram showing the platelet mean fluorescence intensity (MFI; the shadow part shown the SD) in function of time upon laser injury when the animals received a control treatment (top) or the H001 antibody (bottom, dose of 5 µg). (**Fig. 12B**) Histogram showing the platelet mean fluorescence intensity (MFI) in function of time upon laser injury when the animals received a control treatment (top) or the H002 antibody (bottom, dose of 5 µg). (**Fig. 12C**) Histogram showing the platelet mean fluorescence intensity (MFI) in function of time upon laser injury. The animals received a control treatment (top) or the H002 antibody (bottom, dose of 10 µg) 24 hours before injury.

Figures 13A to C show that while the injected humanized Fab H001 is able to bind to platelets *in vivo*, it did not cause an increase in the expression of P-selectin or phosphatidylserine (PS). Results are shown as flow cytometry results for (**Fig. 13A**) platelets (**Fig. 13B**) P-selectin and (**Fig. 13C**) phosphatidylserine.

Figure 14A and B show that the humanized Fabs H001 and H002 antibodies prevented or prolonged vessel occlusion in a FeCl₃-induced carotid artery thrombosis model *in vivo*. (**Fig. 14A**) Representative photographs showing mice carotid flow (mL/min) when the animals received a control treatment (top panel), or the Fab H001 antibody (middle panel, dose of 10 µg) treatment, or the Fab H002 antibody (bottom panel, dose of 10 µg) treatment 5 minutes before injury. The arrows indicated time when vessel occlusion. (**Fig. 14B**) Histogram showing the time to vessel occlusion (in minutes) in function of the antibody or the doses used. * P<0.05, # P<0.05, ** P<0.01.

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Figure 15A to C show that the humanized Fabs H001 and H002 dramatically decreased the infarct size of the ischemic brain without increasing the risk of intracerebral hemorrhage in an cerebral ischemia and reperfusion injury mouse model (transient middle cerebral artery occlusion (tMCAO) model). (**Fig. 15A**) Histogram showing the ischemic brain infarct area in function of the antibody or the doses used immediately after induction of tMCAO. (**Fig. 15B**) Histogram showing the ischemic brain infarct area in function of the humanized Fab H002 (dose of 100 µg) treatment after 1 hour of tMCAO. (**Fig. 15C**) Representative photographs of multiple 2 mm-thick coronal brain sections cut from a whole brain 24 hours after induction of tMCAO in a shame group (without inserting filament), or in a control group treated with a PBS control (200 µL), or in the treatment groups treated with the humanized Fab H001 antibody (100 µg/mouse), or H002 antibody (100 µg/mouse and 50 µg/mouse), respectively immediately after tMCAO. The white color area indicates the infarct brain. * P<0.05, ** P<0.01.

Figure 16A to B show that prophylactic treatment of ADAMTS13^{-/-} mice with the humanized Fab H001 or the humanized C100-scFv fused with human albumin (C100-scFv-HSA) effectively inhibited ionophore-provoked VWF-mediated microvascular thrombosis in a mouse model of TTP. (**Fig. 16A**) Representative photographs showing platelet thrombus accumulation in ADAMTS13^{-/-} mice with and without H001 or C100-scFv-HSA treatment. ADAMTS13^{-/-} mice were injected with fluorescently labeled platelets from mice of the same genotype, and their mesenteric vessels were then exposed and treated with calcium ionophore to induce VWF secretion. Platelet accumulation in the vessels was monitored microscopically. Sequential images taken at the indicated times after the application of calcium ionophore to ADAMTS13^{-/-} mice (control) or with the prophylactic treatment of H001 or C100-scFv-HSA. (**Fig. 16B**) Histogram showing number of emboli (platelet thrombi larger than 20 µm in diameter) in ionophore-provoked ULVWF-mediated microvascular thrombosis in a mouse model of TTP. (**Fig. 16C**) Histogram showing the time to restore normal blood flow in function of the antibody or the doses used. * P<0.05, ** P<0.01.

Figure 17 shows that the humanized Fabs H001 and H002 antibodies did not induce thrombocytopenia. Results are shown as the platelet counts change (in %) in function of time (hours) and antibody treatment: IVIG (○), NIT-B1 (◆), H001 (△) or H002 (▼).

Figure 18 shows that the humanized Fabs H001 and H002 antibodies did not prolong the bleeding time whereas the murine NIT-B markedly increase the bleeding time. Results are shown as the bleeding time (in minutes) in function of treatment and dose (as indicated below the X axis).

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Figure 19A to D show that the humanized C100-scFv and humanized C100-scFv fused with human albumin (C100-scFv-HSA) bind to (**Fig. 19A**) wild-type mouse platelets but not to (**Fig. 19B**) GPIb α -/- mouse platelets, as well as bind to (**Fig. 19C**) human platelets at dose indicated. The “*” shown on Figures 19A and C indicated the control signal.

5 **Figure 20** shows standard aggregometry traces indicating that the humanized C100-scFv inhibited platelet aggregation induced by ristocetin.

Figure 21 shows standard aggregometry traces indicating that the humanized C100-scFv did not induce platelet activation in platelet-rich plasma.

10 **Figure 22** shows that the humanized C100-scFv-HSA inhibited thrombus formation from human whole blood at high shear (1200 s⁻¹) rate conditions. Representative photographs showing the platelet thrombus formation after heparinized-human whole blood were perfused for 1, 2, and 3 minutes, which were treated with a control PBS buffer (top panels) and the humanized C100-scFv-HSA (10 μ g/mL, bottom panels) at high shear (1200 s⁻¹) condition.

DETAILED DESCRIPTION

15 *Anti-GPIb α antibodies*

US Patent Serial Number 8,323,652 describes that the murine NIT mAbs (NIT-A1, NIT-B1, and NIT-F1), which were generated by immunizing the GPIb α -deficient BALB/c mice with wild-type platelets, could specifically recognize both the human and the mouse GPIb α , markedly inhibited ristocetin-induced platelet aggregation and thrombus formation. However, 20 as shown in the Examples below, these intact mAbs could induce severe thrombocytopenia. Furthermore, because they are of mouse origin, they are immunogenic in humans.

The present disclosure provides for specific antibodies against the GPIb α polypeptide. The antibodies are considered “specific” to the GPIb α polypeptide because their affinity for the GPIb α polypeptide is higher than for other polypeptides (for example other platelet surface 25 polypeptides). The antibodies of the present disclosure can recognize and bind to the human GPIb α polypeptide (as described in Gene ID: 2811), the mouse GPIb α polypeptide (as described in Gene ID: 110331805 as well as Gene ID: 110304274), the rat GPIb α polypeptide (as described in Gene ID: 691992), the monkey GPIb α polypeptide (Gene ID: 721584), the dog GPIb α polypeptide (Gene ID: 403638) and/or the rabbit GPIb α polypeptide 30 (Gene ID: 100349951). In an embodiment, the antibodies of the present disclosure can recognize and bind to the human GPIb α polypeptide (as described in Gene ID: 2811), the mouse GPIb α polypeptide (as described in Gene ID: 110331805 as well as Gene ID: 110304274), the rat GPIb α polypeptide (as described in Gene ID: 691992), the monkey

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GP1b α polypeptide (Gene ID: 721584), the dog GP1b α polypeptide (Gene ID: 403638) and/or the rabbit GP1b α polypeptide (Gen ID: 100349951).

In an embodiment, the humanized antibodies of the present disclosure have a dissociation constant (K_D) with the human GP1b α of 10 μ M, 10 nM, 10 pM or lower. In some
5 embodiments, the dissociation constant (K_D) of the humanized antibodies with the human GP1b α is 9, 8, 7, 6, 5, 4, 3, 2, 1 μ M or lower. In some embodiments, the dissociation constant (K_D) of the humanized antibodies with the human GP1b α is 9, 8, 7, 6, 5, 4, 3, 2, 1 nM or lower. In some embodiments, the dissociation constant (K_D) of the humanized antibodies with the human GP1b α is 9, 8, 7, 6, 5, 4, 3, 2, 1 pM or lower.

10 The antibodies of the present disclosure are "humanized" antibodies because they include both a region derived from a human antibody or immunoglobulin and a region derived from a non-human antibody or immunoglobulin. The action of humanizing an antibody consists in substituting a portion of a non-human antibody with a corresponding portion of a human
15 antibody. For example, a humanized antibody as used herein could comprise a non-human origin variable region (such as a region derived from a murine (e.g., mouse) antibody) capable of specifically recognizing GP1b α and a human framework region derived from a human antibody. In another example, the humanized immunoglobulin can comprise a heavy chain and a light chain, wherein the light chain comprises one or more complementarity determining regions (or CDR) derived from an antibody of non-human origin which binds to
20 the GP1b α polypeptide and a framework region (or FR) derived from a light chain of human origin, and the heavy chain comprises a complementarity determining region derived from an antibody of non-human origin which binds to the GP1b α polypeptide and a framework region derived from a heavy chain of human origin. A "complementary determining region" or "CDR" refers to a region of the immunoglobulin located in the variable parts of the polypeptide and
25 involved in specifically binding the epitope. The combination of CDRs constitutes the paratope of the antibody.

The human region of the humanized antibody can be derived from an IgG, IgM, IgA, IgE or IgD isotype. In some embodiments, the human region of the humanized antibody can be derived from an IgG isotype, for example, from the IgG1, IgG2, IgG3 or IgG4 subclass. In
30 some specific embodiments, the human region of the humanized antibody can be derived from the IgG1 subclass. As indicated below, because the humanized antibodies are also monovalent antibodies, the human region of the humanized antibody can include a heavy chain which is derived from a CH₁ region and/or a V_H region (excluding the CDRs) and exclude the CH₂ and/or CH₃ region. The human region of the humanized antibodies can
35 include a light chain which is derived from a C_L region and/or a V_L region (excluding the

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CDRs). The human region of the humanized antibodies includes a light chain which can be of the kappa or lambda type. In a specific embodiment, the human region of the humanized antibody includes a light chain which is from the kappa type.

The humanized antibodies of the present disclosure do not include (e.g., lack) a Fc moiety. For example, the humanized antibody moiety is the fragment antigen-binding region F(ab)₂ of a multivalent antibody. The F(ab)₂ fragment is a dimer of two molecular entities (a light chain fragment and a heavy chain fragment), consists of a single antigen-binding site and comprises one constant and one variable domain from each heavy and light chain of the antibody which are associated to one another by disulfide bonds. Each chain of the F(ab)₂ includes three V_L and three V_H domains. The F(ab)₂ antibody moiety can be fully or partially glycosylated, when compared to the parent multivalent antibody it can be derived from.

In some embodiments, the antibodies of the present disclosure are "monovalent" antibodies. As used in the context of the present disclosure, a "monovalent" antibody contains a single antigen binding site. The monovalent antibody moiety has no more than one variable light domains (V_L) associated (covalently or not) and no more than one corresponding variable heavy domains (V_H). This is different with multivalent full-length antibodies which comprises at least two antigen binding sites and more than one V_H and more than one V_L domains. The monovalent antibody moiety can be fully or partially glycosylated, when compared to the parent multivalent antibody it can be derived from. In some instances, the monovalent antibody moiety is not glycosylated. The monovalent antibody moiety is capable of competing for the binding site that is recognized by the corresponding multivalent antibody (e.g., NIT-B1 in some embodiments). The monovalent antibody moiety does not include the crystallizable fragment (Fc fragment) of the multivalent antibody it is derived from.

In some instances, the monovalent antibody is a single-chain variable fragment (scFv) derived from one or more multivalent antibody. The scFv is single molecular entity (a fusion protein) consisting of a single antigen-binding region and having no more than one V_H and no more than one V_L domains from a multivalent antibody which are connected with a linker (e.g., usually a short peptide linker). As such, the scFv consists of a single antigen-binding region and comprises one V_H and one V_L domains. The scFv can be obtained from screening a synthetic library of scFvs, such as, for example, a phage display library of scFvs. The scFvs of the present disclosure can include, for example, one or more GGGGS (SEQ ID NO: 92) linker between the V_H and the V_L domains. In some embodiments, the carboxy terminus of the V_L domain can be linked to the amino terminus of the V_H domain. In another embodiment, the carboxy terminus of the V_H domain can be linked to the amino terminus of the V_L domain. In some embodiments, the scFvs of the present disclosure can include a

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purification tag (such as, for example a 6 X His tag) which can be removed once the scFv is purified. In some additional embodiments, the scFv can be (covalently) associated with a carrier protein to form chimeric protein. In such embodiments, the carrier protein can be linked at the amino terminus or the carboxy terminus of the scFv. In some embodiments, the
5 scFv does not include a purification tag or has been processed to remove a purification tag.

In other instances, the monovalent antibody is the fragment antigen-binding region (Fab) of a multivalent (and in some embodiments, monoclonal) antibody. The Fab fragment comprises two molecular entities (a light chain fragment and a heavy chain fragment), consists of a single antigen-binding site and comprises one constant and one variable domain from each
10 heavy and light chain of the antibody which are associated to one another by disulfide bonds. The Fab includes a single V_L and a single V_H domain.

In further instances, the monovalent antibody is a single domain antibody or a nanobody. The single domain antibody includes a single monomeric variable antibody domain comprising at least three complementary determining regions (CDRs). The single domain
15 antibodies can be obtained from camelids (e.g., V_{HH} antibodies), from fish (e.g. V_{NAR} antibodies) or from phage display. The single domain antibodies can be derived from a heavy chain or a light chain. The single domain antibodies can be humanized.

The antibodies of the present disclosure can be capable of preventing platelet activation and aggregation. The expression "capable of preventing platelet activation and aggregation"
20 refers to the ability of the humanized antibodies of the present disclosure to, in the presence of a platelet and a platelet agonist, avoid activating the platelet and aggregating platelets. Platelet activation occurs primarily during the initiation of the hemostasis or thrombosis. Upon activation, platelets change their shape and release the content of their granules. Activated platelets modulate the expression of their membrane proteins (e.g., P-selectin), lipids (e.g.,
25 phosphatidylserine) and conformational changes of platelet $\alpha IIb\beta 3$ integrin that results in platelet aggregation. Platelet activation and aggregation can be measured, for example, by determining the shape of the platelet, the level of aggregation of platelets (using for example an aggregometer), the expression of surface protein or lipids, etc. Platelets can be activated with the following agonists (activators), thrombin, ADP, collagen and others. Ristocetin can
30 also cause von Willebrand factor to bind to platelet receptor GPIIb α . In order to determine if a humanized antibody prevents platelet activation and aggregation, platelets (which can be obtained in the form of platelet-rich plasma or gel-filtered platelets for example) can be placed in contact with the humanized antibody first and then with the agonist. Then, it should be determined, by methods known in the art, if the platelets are activated/aggregated or not.

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Antibodies preventing platelet activation and aggregation are considered to be antibodies of the present disclosure.

In addition, the humanized antibodies of the present disclosure can lack the ability to induce platelet activation. The expression "lack the ability to induce platelet activation" refers to one of the properties of the humanized antibodies of the present disclosure, namely that they do not activate platelets in the absence of a known platelet agonist. In order to determine if a humanized antibody lacks the ability to induce platelet activation, platelets (which can be obtained in the form of platelet-rich plasma or gel-filtered platelets for example) can be placed in contact with the antibody (in the absence of a known platelet agonist) and then, it should be determined, by methods known in the art, if the platelets are activated or not. Antibodies that fail to induce platelet activation are considered to be antibodies of the present disclosure.

The antibodies of the present disclosure can lack the ability to induce thrombocytopenia. The expression "lack the ability to induce thrombocytopenia" refers to one of the properties of the humanized antibodies of the present disclosure, namely that they do not cause a substantial and pathological deficiency in the total number of platelets. In humans, thrombocytopenia requiring emergency treatment is a count below 50 000 platelets per μL of blood. In order to determine if a humanized antibody lacks the ability to induce thrombocytopenia, it is administered to a test subject (a mouse for example) and the level of platelets is monitored using techniques known in the art to determine if the antibody causes a decrease (and if so a substantive or pathological decrease) in platelet count. Antibodies that fail to induce thrombocytopenia are considered to be antibodies of the present disclosure.

The antibodies of the present disclosure can lack the ability to prolong bleeding time (at therapeutic doses). The expression "lack the ability to prolong bleeding time" refers to one of the properties of the humanized antibodies of the present disclosure, namely that they do not cause a substantial and pathological increasing the time it takes to stop bleeding. In order to determine if a humanized antibody lacks the ability to prolong bleeding time, a cut (of standardized width and depth) is made on the tail of a test subject (a mouse for example), the time it takes for the bleeding to stop (e.g., cessation of blood flow for a minimal amount of time) is determined using techniques known in the art (in some embodiments, the Ivy method or Duke method) and compared to a standard to ascertain if the antibody causes an increase (and if so a substantive increase) in bleeding time. Antibodies that fail prolonging bleeding time at doses indicated are considered to be antibodies of the present disclosure.

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The antibodies of the present disclosure can also be capable of antagonizing the biological activity of the GPIb α polypeptide. The GPIb α polypeptide is a platelet surface membrane glycoprotein serving as a receptor for the von Willebrand factor (VWF), thrombin as well as other ligands. By antagonizing its biological activity, the antibodies of the present disclosure
5 can thus be used to limit or prevent platelet activation and aggregation (especially under high shear conditions).

Antibodies of the present disclosure can be derived from monoclonal antibodies. Antibodies which are specific for a single epitope on the GPIb α polypeptide are considered as monoclonal antibodies (also referred to as mAbs). In some embodiments, monoclonal
10 antibodies are produced from a single clone of an immune cell. Monoclonal antibodies can be produced by techniques known in the art, such as by using cell culture by fusing a myeloma cell to a spleen cell from a subject (such as a mouse or a human) which has been immunized with an antigen comprising the epitope of the GPIb α polypeptide. Monoclonal antibodies can also be obtained phage display by screening library of monoclonal antibodies
15 using an antigen comprising the epitope of the GPIb α polypeptide. Additional techniques for making monoclonal antibodies include, but are not limited to single B cell culture, single cell amplification from B cell populations. Monoclonal antibodies of the present disclosure can be from various origins (e.g., mouse or human for example) and can include two identical light chains and two identical heavy chains, wherein each chain comprises three CDRs.
20 Monoclonal antibodies can be from any isotype, including, but not limited to immunoglobulin A (IgA), IgD, IgE, IgG (including subtypes IgG1, IgG2, IgG3 or IgG4) or IgM. Monoclonal antibodies can be, in an embodiment, from the IgG isotype.

In an embodiment, the antibodies of present disclosure have at least one complementary determining region comprising or consisting essentially of the amino acid sequence of SEQ
25 ID NO: 37, 38, 39, 65, 66 or 67, variant thereof or fragments thereof. In the context of the present disclosure, and especially when referred to the amino acid sequence of CDR, the expression "consisting essentially of" indicates that the CDR necessarily comprises the amino acid sequence of SEQ ID NO: 37, 38, 39, 65, 66 or 67, but that additional, non-essential, amino acid residues can be added at the amino or the carboxyl end of those
30 sequences (as long as these amino acid residues do not substantially modify the affinity of the antibody for the GPIb α polypeptide or its ability to antagonize the biological activity of the GPIb α polypeptide).

In an embodiment, the antibodies of the present disclosure have at least two complementary determining region comprising or consisting essentially of the amino acid sequence of SEQ
35 ID NO: 37, 38, 39, 65, 66 or 67, variant thereof or fragments thereof. In still another

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embodiment, the antibodies of the present disclosure have at least three complementary determining region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 37, 38, 39, 65, 66 or 67, variant thereof or fragments thereof. In yet another embodiment, the antibodies of the present disclosure have at least four complementary determining region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 37, 38, 39, 65, 66 or 67, variant thereof or fragments thereof. In still another embodiment, the antibodies of the present disclosure have at least five complementary determining region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 37, 38, 39, 65, 66 or 67, variant thereof or fragments thereof. In still another embodiment, the antibodies of the present disclosure have complementary determining region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 37, 38, 39, 65, 66 and 67, variant thereof or fragments thereof.

In some embodiments, the antibodies of the present disclosure have the complementary determining region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 37, 38 and 39 (including variants and fragments) as well as at least one complementary determining region comprising or consisting essentially or SEQ ID NO: 65, 66 or 67 (including variants and fragments). In some additional embodiments, the antibodies of the present disclosure have the complementary determining region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 37, 38 and 39 (including variants and fragments) as well as at least two complementary determining region comprising or consisting essentially or SEQ ID NO: 65, 66 or 67 (including variants and fragments). In some further embodiments, the antibodies of the present disclosure have the complementary determining region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 65, 66 and 67 (including variants and fragments) as well as at least one complementary determining region comprising or consisting essentially or SEQ ID NO: 37, 38 or 39 (including variants and fragments). In some additional embodiments, the antibodies of the present disclosure have the complementary determining region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 65, 66 and 67 (including variants and fragments) as well as at least two complementary determining region comprising or consisting essentially or SEQ ID NO: 37, 38 or 39 (including variants and fragments).

The antibody of the present disclosure can include a functional variant of a CDR having the amino acid sequence of SEQ ID NO: 37, 38, 39, 65, 66 or 67. A variant CDR comprises at least one amino acid difference when compared to the amino acid sequence of the CDR. As used herein, a variant refers to alterations in the amino acid sequence that do not adversely

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affect the biological functions of the antibody (e.g., providing specificity and affinity towards the GPIIb α polypeptide). In some embodiments, the overall charge, structure or hydrophobic-hydrophilic properties of the antibody can be altered without adversely affecting a biological activity. Accordingly, the amino acid sequence of the CDR can be altered, for example to
5 render the antibody more hydrophobic or hydrophilic, without adversely affecting the biological activities of the antibody. The CDR variants have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the CDRs described herein. The term "percent identity", as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by
10 comparing the sequences. The level of identity can be determined conventionally using known computer programs. Identity can be readily calculated by known methods, including but not limited to those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1993); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular
15 Biology (von Heinje, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Stockton Press, NY (1991). Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer
20 programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, Wis.). Multiple alignments of the sequences disclosed herein were performed using the Clustal method of alignment (Higgins and Sharp (1989) CABIOS. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PEN ALT Y= 10). Default
25 parameters for pairwise alignments using the Clustal method were KTUPLB 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

The CDR variants may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one
30 encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group. A "variant" of the CDR can be a conservative variant or an allelic variant.

The antibody of the present disclosure can include a functional fragment of a CDR having the amino acid sequence of SEQ ID NO: 37, 38, 39, 65, 66 or 67. A fragment of a CDR
35 comprises at least one less amino acid residue compared to the amino acid sequence of the

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CDR. The CDR fragments comprise some consecutive amino acid residues of the CDR of amino acid sequence of SEQ ID NO: 37, 38, 39, 65, 66 or 67. The CDR fragments have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the CDRs described herein.

5 In another embodiment, the antibodies of the present disclosure comprise a heavy chain and the heavy chain comprises at least one CDR comprising or consisting essentially of an amino acid sequence of SEQ ID NO: 37, 38 or 39, functional variants thereof and functional fragments thereof. In a further embodiment, the heavy chain comprises at least two CDRs
10 each comprising or consisting essentially of a distinct amino acid sequence from the following: SEQ ID NO: 37, 38 or 39, functional variants thereof and functional fragments thereof. In still a further embodiment, the heavy chain comprises three CDRs each comprising or consisting essentially of a distinct amino acid sequence from the following: SEQ ID NO: 37, 38 and 39 functional variants thereof and functional fragments thereof.

In another embodiment, the heavy chain includes a CH1 region of a human IgG1 antibody
15 and comprises the amino acid sequence of SEQ ID NO: 40, 47, 54 or 61, functional variants thereof as well as functional fragments thereof. As used in the context of the present disclosure, a functional variant of a CH1 region of a human IgG1 antibody refers to alterations in the amino acid sequence that do not adversely affect the biological functions of the antibody (*e.g.*, providing specificity and affinity towards the GPIIb α polypeptide). In an
20 embodiment, the functional variant of the CH1 region of the human IgG1 antibody has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the CH1 region described herein (such as, for example, those having the amino acid sequence of SEQ ID NO: 40, 47, 54 or 61). As also used in the context of the present disclosure, a functional fragment of a CH1 region of a human IgG1 antibody refers to
25 comprises at least one less amino acid residue compared to the amino acid sequence of the CH1 region of the human IgG1 antibody that do not adversely affect the biological functions of the antibody (*e.g.*, providing specificity and affinity towards the GPIIb α polypeptide). In an embodiment, the functional fragment of the CH1 region of the human IgG1 antibody has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%
30 identity to the CH1 region described herein (such as, for example, those having the amino acid sequence of SEQ ID NO: 40, 47, 54 or 61).

In some embodiments, the heavy chain comprises or consists essentially of the amino acid sequence of SEQ ID NO: 36, 43, 50 or 57, functional variants thereof and functional fragments thereof. In the context of the present disclosure, and especially when referred to
35 the amino acid sequence of the heavy chain, the expression "consisting essentially of"

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indicates that the heavy chain necessarily comprises the amino acid sequence of SEQ ID NO: 36, 43, 50 or 57, but that additional, non-essential, amino acid residues can be added at the amino or the carboxyl end of those sequences (as long as these amino acid residues do not substantially modify the affinity of the antibody for the GPIIb α polypeptide or its ability to antagonize the biological activity of the GPIIb α polypeptide). As used in the context of the present disclosure, a functional variant of the heavy chain antibody refers to alterations in the amino acid sequence that do not adversely affect the biological functions of the antibody (e.g., providing specificity and affinity towards the GPIIb α polypeptide). In an embodiment, the functional variant of the heavy chain has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the heavy chain described herein (such as, for example, those having the amino acid sequence of SEQ ID NO: 36, 43, 50 or 57). As also used in the context of the present disclosure, a functional fragment of the heavy chain comprises at least one less amino acid residue compared to the amino acid sequence of the heavy chain that do not adversely affect the biological functions of the antibody (e.g., providing specificity and affinity towards the GPIIb α polypeptide). In an embodiment, the functional fragment of the heavy chain has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the heavy chain described herein (such as, for example, those having the amino acid sequence of SEQ ID NO: 36, 43, 50 or 57).

In another embodiment, the antibody comprises a light chain and the light chain comprises at least one CDR comprising or consisting essentially of an amino acid sequence of SEQ ID NO: 65, 66 or 67, functional variants thereof and functional fragments thereof. In a further embodiment, the light chain comprises at least two CDRs each comprising or consisting essentially of a distinct amino acid sequence from the following: SEQ ID NO: 65, 66 or 67, functional variants thereof and functional fragments thereof. In still a further embodiment, the light chain comprises three CDRs each comprising or consisting essentially of a distinct amino acid sequence from the following: SEQ ID NO: 65, 66 and 67, functional variants thereof and functional fragments thereof.

In another embodiment, the light chain includes a human IgG1 kappa chain C region which can have, for example, the amino acid sequence of SEQ ID NO: 68, 75, 82, or 89, the variant thereof or the fragment thereof. As used in the context of the present disclosure, a functional variant of a human IgG1 kappa chain C region refers to alterations in the amino acid sequence that do not adversely affect the biological functions of the antibody (e.g., providing specificity and affinity towards the GPIIb α polypeptide). In an embodiment, the functional variant of the human IgG1 kappa chain C region has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the a human IgG1 kappa

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chain C region described herein (such as, for example, those having the amino acid sequence of SEQ ID NO: 68, 75, 82 or 89). As also used in the context of the present disclosure, a functional fragment of a human IgG1 kappa chain C region refers to comprises at least one less amino acid residue compared to the amino acid sequence of the a human
5 IgG1 kappa chain C region that do not adversely affect the biological functions of the antibody (e.g., providing specificity and affinity towards the GPIIb α polypeptide). In an embodiment, the functional fragment of the human IgG1 kappa chain C region has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the CH1 region described herein (such as, for example, those having the amino acid
10 sequence of SEQ ID NO: 68, 75, 82 or 89).

In another embodiment, the light chain comprises or consists essentially of the amino acid sequence of SEQ ID NO: 64, 71, 78 or 85, functional variants thereof and functional fragments thereof. In the context of the present disclosure, and especially when referred to the amino acid sequence of a light chain, the expression "consisting essentially of" indicates
15 that the light chain necessarily comprises the amino acid sequence of SEQ ID NO: 64, 71, 78 or 85, but that additional, non-essential, amino acid residues can be added at the amino or the carboxyl end of those sequences (as long as these amino acid residues do not substantially modify the affinity of the antibody for the GPIIb α polypeptide or its ability to antagonize the biological activity of the GPIIb α polypeptide). As used in the context of the
20 present disclosure, a functional variant of a light chain antibody refers to alterations in the amino acid sequence that do not adversely affect the biological functions of the antibody (e.g., providing specificity and affinity towards the GPIIb α polypeptide). In an embodiment, the functional variant of the light chain has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the light chain described herein (such as, for
25 example, those having the amino acid sequence of SEQ ID NO: 64, 71, 78 or 85). As also used in the context of the present disclosure, a functional fragment of a light chain comprises at least one less amino acid residue compared to the amino acid sequence of the heavy chain that do not adversely affect the biological functions of the antibody (e.g., providing specificity and affinity towards the GPIIb α polypeptide). In an embodiment, the functional
30 fragment of the light chain has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the light chain described herein (such as, for example, those having the amino acid sequence of SEQ ID NO: 64, 71, 78 or 85).

In some embodiments, the heavy chain comprises or consists essentially of the amino acid sequence of SEQ ID NO: 36, 43, 50 or 57, functional variants thereof and functional
35 fragments thereof. In the context of the present disclosure, and especially when referred to

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the amino acid sequence of heavy chain, the expression "consisting essentially of" indicates that the CDR necessarily comprises the amino acid sequence of SEQ ID NO: 36, 43, 50 or 57, but that additional, non-essential, amino acid residues can be added at the amino or the carboxyl end of those sequences (as long as these amino acid residues do not substantially modify the affinity of the antibody for the GPIIb α polypeptide or its ability to antagonize the biological activity of the GPIIb α polypeptide). As used in the context of the present disclosure, a functional variant of heavy chain antibody refers to alterations in the amino acid sequence that do not adversely affect the biological functions of the antibody (*e.g.*, providing specificity and affinity towards the GPIIb α polypeptide). In an embodiment, the functional variant of the heavy chain has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the heavy chain described herein (such as, for example, those having the amino acid sequence of SEQ ID NO: 36, 43, 50 or 57). As also used in the context of the present disclosure, a functional fragment of a heavy chain comprises at least one less amino acid residue compared to the amino acid sequence of the heavy chain that do not adversely affect the biological functions of the antibody (*e.g.*, providing specificity and affinity towards the GPIIb α polypeptide). In an embodiment, the functional fragment of the heavy chain has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the heavy chain described herein (such as, for example, those having the amino acid sequence of SEQ ID NO: 36, 43, 50 or 57).

In yet another embodiment, the antibody can comprise both a heavy chain and the light chain. In such embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 36, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 64, the variant thereof or the fragment thereof. In another embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 36, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 64, the variant thereof or the fragment thereof. In still another embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 36, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 71, the variant thereof or the fragment thereof. In yet another embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 36, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 78, the variant thereof or the fragment thereof. In another embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 36, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 85, the variant thereof or the fragment thereof. In still another embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 43, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 64, the variant thereof or the fragment thereof. In yet another embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 43, the

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variant thereof or the fragment thereof and the light chain of SEQ ID NO: 71, the variant thereof or the fragment thereof. In yet a further embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 43, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 78, the variant thereof or the fragment thereof. In a further embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 43, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 85, the variant thereof or the fragment thereof. In a further embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 50, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 64, the variant thereof or the fragment thereof. In a further embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 50, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 71, the variant thereof or the fragment thereof. In yet a further embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 50, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 78, the variant thereof or the fragment thereof. In still another embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 50, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 85, the variant thereof or the fragment thereof. In an embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 57, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 64, the variant thereof or the fragment thereof. In still a embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 57, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 71, the variant thereof or the fragment thereof. In yet another embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 57, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 78, the variant thereof or the fragment thereof. In still a further embodiment, the humanized antibody can have the heavy chain of SEQ ID NO: 57, the variant thereof or the fragment thereof and the light chain of SEQ ID NO: 85, the variant thereof or the fragment thereof.

The heavy and light chains of the antibodies of the present disclosure can include a leader sequence which, upon secretion from the cell, is cleaved. For example, the amino acid sequence of SEQ ID NO: 35 includes the amino acid sequence of SEQ ID NO: 36 as well as additional amino acid residues at the N terminus which act as a leader sequence. The leader sequence which can be included in the heavy and/or light chain includes, but are not limited to the amino acid sequence of SEQ ID NO: 91.

In some embodiment, the antibodies of the present disclosure may be further modified or designed into a chimeric protein (comprising a carrier protein) to increase, amongst other things, their circulation half-life. The humanized antibody moiety can be associated (directly

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or indirectly with the linker, such as, for example one or more GGGGS (SEQ ID NO : 92) linker) to the carrier at any amino acid residue(s), provided that the association does not impede the humanized antibody moiety from binding to GPIIb α and inhibiting its biological activity. In an embodiment, the linker includes three copies of the GGGGS (SEQ ID NO : 92) linker. In another embodiment, the linker includes four copies of the GGGGS (SEQ ID NO : 92) linker. In some instances, the linker (when present) or the carrier is associated to one or more amino acid residue(s) of the humanized antibody moiety that is (are) not involved in specifically binding to GPIIb α and inhibiting its biological activity. In some instances, the linker or the carrier is associated to a single amino acid residue of the humanized antibody moiety.

10 The linker or the carrier can be associated with any amino acid residue of the humanized antibody moiety, including the amino acid residue located at the amino-terminus of the humanized antibody moiety or at the carboxyl-terminus of the humanized antibody moiety. In some embodiments, the carrier protein can be located upstream (at the amino end) or downstream (at the carboxy end) of the humanized antibody moiety. In instances in which

15 the linker and the carrier are also of proteinaceous nature, the humanized antibody moiety can be associated to any amino acid residue of the linker or the carrier, including the amino acid residue located at the amino-terminus of the linker or the carrier or the amino acid residue located at the carboxyl-terminus of the linker or the carrier. In an embodiment, the amino acid residue located at the amino-terminus of the linker or the carrier is associated to

20 the amino acid residue located at the carboxyl-terminus of the humanized antibody moiety. In still another embodiment, when the linker is present and is of proteinaceous nature, its amino terminus is associated to the carboxyl terminus of humanized antibody and its carboxyl terminus is associated with the amino terminus of the carrier. In an embodiment, the carrier protein is albumin (e.g., human serum albumin for example). In an embodiment, the carrier

25 comprises one or more further antibody or an antibody fragment.

In instances where a covalent association is sought between the humanized antibody moiety and the carrier, the association between the two entities can be a peptidic bond. Such embodiment is especially useful for chimeric proteins wherein the at least two entities are both proteinaceous and are intended to be produced as a fusion protein in an organism

30 (prokaryotic or eukaryotic) using a genetic recombinant technique. Alternatively, the covalent association between the two moieties can be mediated by any other type of chemical covalent bounding. In some instances, the chimeric proteins are designed so as not to be susceptible of being cleaved into the two moieties in the general circulation (for example in plasma).

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As indicated above, the association between the two entities (e.g., humanized antibody moiety and carrier moiety) can be non-covalent. Exemplary non-covalent associations include, but are not limited to the biotin-streptavidin/avidin system. In such system, a label (biotin) is covalently associated to one entity/moiety while a protein (streptavidin or biotin) is covalently associated with the other entity/moiety. In such embodiment, the biotin can be associated to the humanized antibody moiety or to the carrier, providing that the other entity in the system is associated with streptavidin or avidin.

In a further system of non-covalent association, the first entity is designed to be non-covalently associated to the second entity only upon its administration into the intended recipient. This embodiment is especially useful when the carrier is a protein present in the blood of the recipient. For example, the humanized antibody moiety may be associated (in a covalent or a non-covalent fashion) with a second antibody, a lectin or a fragment thereof (referred to herein as an antibody-derived linker) which is capable of non-covalently binding the carrier once administrated to the intended recipient. For example, the second antibody, lectin or fragment thereof can be specific for any blood/plasma protein present in the intended recipient (such as, for example, serum albumin, immunoglobulins fragments (provided that these fragments do not directly bind the activating Fc receptor or cause the chimeric protein to simultaneously bind to more than one site on the activating Fc receptor), alpha-1-acid glycoprotein, transferrin, or lipoproteins). The second antibody, lectin or fragment thereof can be associated, preferably in a covalent manner, with the humanized antibody moiety at any amino acid residue of the humanized antibody moiety, but preferably at the amino- or carboxyl-end of the humanized antibody moiety. In such embodiment, the second antibody, lectin or fragment thereof is akin to a linker between the humanized antibody moiety and the carrier. Upon the administration of this embodiment of the humanized antibody moiety in the recipient, the carrier (a blood or plasma protein for example) associates with the second antibody, lectin or fragment thereof to form, *in vivo*, the chimeric protein. In a specific embodiment, the second antibody is an antibody specifically recognizing albumin (such as, for example, an antibody specifically recognizing human albumin).

The present disclosure also provides nucleotide molecules encoding the antibodies described herein. The nucleotide molecules can be provided in an isolated form and may be derived from a variety of sources including DNA, cDNA, synthetic DNA, synthetic RNA, derivatives, mimetics or combinations thereof. Such sequences may comprise genomic DNA, which may or may not include naturally occurring introns, genic regions, non-genic regions, and regulatory regions. Moreover, such genomic DNA may be obtained in association with

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promoter regions or poly (A) sequences. The sequences, genomic DNA, or complementary DNA (cDNA) may be obtained in any of several ways. Genomic DNA can be extracted and purified from suitable cells by means well known in the art. Alternatively, mRNA can be isolated from a cell and used to produce cDNA by reverse transcription or other means. The nucleotide molecules described herein are used in certain embodiments of the methods of the present disclosure for production of RNA, proteins or polypeptides, through incorporation into host cells, tissues, or organisms. In an embodiment, the nucleotide molecules can be codon-optimized for expression in a particular host. The nucleotide molecules can include, in some embodiments, one or more promoter sequence and/or one or more terminator sequence. The nucleotide molecules can be included in a vector for expression in a recombinant host. The nucleotide molecules of the present disclosure can include, in some embodiments, the nucleic acid sequence of SEQ ID NO: 41, 48, 55, 62, 69, 76, 83 and/or 90. In an embodiment, the nucleotide sequence of the present disclosure includes the nucleic acid sequence of SEQ ID NO: 41 and 69, 41 and 76, 41 and 83 or 41 and 90. In another embodiment, the nucleotide sequence of the present disclosure includes the nucleic acid sequence of SEQ ID NO: 48 and 69, 48 and 76, 48 and 83 or 48 and 90. the nucleotide sequence of the present disclosure includes the nucleic acid sequence of SEQ ID NO: 55 and 69, 55 and 76, 55 and 83 or 55 and 90. the nucleotide sequence of the present disclosure includes the nucleic acid sequence of SEQ ID NO: 62 and 69, 62 and 76, 62 and 83 or 62 and 90.

Therapeutic uses of the humanized antibodies

Since platelet GPIIb/IIIa and its ligands (such as VWF) interactions have been recognized as important players in the pathogenesis of diverse diseases, the humanized antibodies can be used in the prevent and/or treatment of ischemic stroke, acute myocardial infarction, restenosis, angina, acute coronary syndrome, atherothrombosis, vascular inflammation, venous thrombosis, peripheral vascular disease, thrombotic thrombocytopenic purpura, sepsis and/or tumor metastasis. The humanized antibody or the chimeric protein can be used in a subject having platelets being specifically recognized by the humanized antibody (or the humanized antibody moiety of the chimeric protein). As such, the humanized antibody can be used in a mammal subject, such as, for example, a human, a monkey, a mouse, a rabbit and/or a dog, etc.

The present disclosure provides a method for preventing or limiting the physical interaction between GPIIb/IIIa and its cognate ligand. The method comprises contacting the humanized antibody, the chimeric protein or the pharmaceutical composition described herein with a platelet (which expresses on its surface GPIIb/IIIa) under conditions to allow the binding of the

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humanized antibody/humanized antibody moiety with GPIb α . As shown in the Examples below, the humanized antibodies and the chimeric proteins of the present disclosure are capable of binding to GPIb α and antagonizing its biological activity under low and high shear stress. As such, the method can be used to bind to GPIb α irrespective of the shear stress
5 applied. The method can be used *in vitro* or *in vivo* in a subject in need thereof. The method can be used in low or high shear rates.

When it is sought to prevent the interaction between GPIb α and its ligands (such as VWF), the humanized antibody or the chimeric protein can be used prior to the contact between GPIb α and its ligand. As such, the platelet is first contacted with the humanized antibody
10 (which is optionally presented as a chimeric protein or a pharmaceutical composition) before its ligand is placed or is found in vicinity of the platelet. In such embodiment, it is understood that the binding of the humanized antibody of the present disclosure will prevent the physical association of GPIb α with its ligand and, ultimately, prevent or limit platelet activation and aggregation.

15 When it is sought to limit the interaction between GPIb α and its ligands (such as VWF), the humanized antibody can be used at the same time or after the contact between GPIb α and its ligand has occurred. As such, the platelet is contacted with the humanized antibody (which is optionally presented as a chimeric protein or a pharmaceutical composition) simultaneously or after its ligand is placed or is found in vicinity of the platelet. In such
20 embodiment, it is understood that the binding of the humanized antibody of the present disclosure will limit the physical association of GPIb α with its ligand and, in some embodiments, prevent or limit platelet activation and aggregation.

The humanized antibody (optionally in a chimeric form or in a pharmaceutical composition) can be used to prevent, treat or alleviate the symptoms associated with pathological
25 thrombosis in a subject in need thereof. Since the humanized antibody of the present disclosure can prevent platelet activation and aggregation (at least in the Examples below), they can be used to prevent pathological thrombosis in a subject susceptible to pathological thrombosis. In addition, since the humanized antibody of the present disclosure do not induce thrombocytopenia or prolong bleeding, they are safer to use (than for example, the
30 original monoclonal antibody they are derived from). As used in the context of the present disclosure, the term "pathological thrombosis" refers to a condition in which a thrombus (a blood clot) forms in a blood vessel and causes a damage to the surrounding tissues. The pathological thrombosis can occur in a vein or an artery. The pathological thrombus can occur or be observed in a cavernous sinus, a renal vein, a deep vein or in a lung (pulmonary
35 embolism). In some embodiments, the humanized antibody or the chimeric protein is used to

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prevent, treat or alleviate the symptoms associated with pathological thrombosis under high shear stress conditions. In the vicinity of an occluded or partially occluded vessel, shear stress is high and the interaction between GPIIb/IIIa and VWF is critical for vessel occlusion.

5 In embodiments in which it is warranted to prevent the formation or the growth of a thrombus, the humanized antibody or the chimeric protein can be used in subject at risk of forming or growing a thrombus. In an embodiment, the method can include determining if, prior to administration of the antibody, the subject is at risk of forming or growing a thrombus (with methods and assays known in the art). The humanized antibody can be used in subject which has previously been determined to be at risk of forming or growing a thrombus. In
10 another embodiment, the method can include determining, after the administration of at least one dose of the humanized antibody or the chimeric protein, if the subject has at least one thrombus and, in some further embodiments, the size of the thrombus. Such determination can help determine if additional doses should be administered to the subject to achieve the desired therapeutic effect.

15 In subjects having a plurality of thrombi, the humanized antibody or the chimeric protein can be used to reduce the size and/or the number of thrombi. In an embodiment, the method can include determining if, prior to administration of the antibody, the subject has one or more thrombus and optionally the size of the thrombus (with methods and assays known in the art). The humanized antibody can be used in a subject which has previously been
20 determined have a plurality of thrombi and optionally the size of the thrombus. In another embodiment, the method can include determining, after the administration of at least one dose of the humanized antibody or the chimeric protein, the presence, number and size of the thrombus. Such determination can help determine if additional doses should be administered to the subject to achieve the desired therapeutic effect.

25 In some embodiments, the methods of the present disclosure includes determining if the subject is at risk of experiencing or has experienced a pathological thrombosis. A positive determination that the subject is at risk of experiencing or has experienced pathological thrombosis is indicative that the subject would benefit from receiving the humanized antibodies of the present disclosure. As such, the methods of the present disclosure can
30 include administering the humanized antibody or the chimeric protein to a subject which has been determined to be at risk of experiencing or has experienced a pathological thrombosis. The humanized antibodies and the chimeric protein of the present disclosure can be used in subjects in which it has been determined that they are at risk of experiencing or has experienced a pathological thrombosis.

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In some further embodiments, the methods of the present disclosure includes determining if the subject is at risk of experiencing or has experienced an ischemic stroke, a thrombotic thrombocytopenic purpura, a myocardial infarct, an acute coronary syndrome, atherothrombosis, a peripheral vascular disease, deep vein thrombosis, sepsis, and/or
5 vascular inflammation. A positive determination that the subject is at risk of experiencing or has experienced an ischemic stroke, a thrombotic thrombocytopenic purpura, a myocardial infarct, an acute coronary syndrome, atherothrombosis, a peripheral vascular disease, deep vein thrombosis, sepsis, and/or vascular inflammation is indicative that the subject would benefit from receiving the humanized antibodies of the present disclosure. As such, the
10 methods of the present disclosure can include administering the humanized antibody to a subject which has been determined to be at risk of experiencing or has experienced an ischemic stroke, a thrombotic thrombocytopenic purpura, a myocardial infarct, an acute coronary syndrome, atherothrombosis, a peripheral vascular disease, deep vein thrombosis, sepsis, and/or vascular inflammation. The humanized antibodies of the present disclosure
15 can be used in subjects in which it has been determined that they are at risk of experiencing or has experienced an ischemic stroke, a thrombotic thrombocytopenic purpura, a myocardial infarct, an acute coronary syndrome, atherothrombosis, a peripheral vascular disease, deep vein thrombosis, sepsis, and/or vascular inflammation.

Because the interaction between GPIIb/IIIa and VWF is important for the dissemination of tumor
20 metastasis, the humanized antibody or the chimeric protein of the present disclosure can be used to reduce or limit tumor metastasis in a subject in need thereof. In some embodiments, the humanized antibody or the chimeric protein can be used to reduce the number of tumor metastasis and/or the size of tumor metastasis. In an embodiment, the tumor metastasis are associated with a liver cancer (such as a liver carcinoma or adenocarcinoma) and the
25 humanized antibody can be used to reduce or limit liver tumor metastasis.

In some embodiments, the methods of the present disclosure includes determining the presence, location and/or the size of the tumor metastasis prior to and/or after having provided one or more dose of the humanized antibodies or of the chimeric proteins. Such assessment can be useful to determine if additional doses of the humanized antibody should
30 be administered to achieve the desired therapeutic result in the subject.

The humanized antibody or the chimeric protein comprising same can be formulated as a pharmaceutical composition for administration with an excipient. An excipient or "pharmaceutical excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more chimeric protein to a
35 subject, and is typically liquid. A pharmaceutical excipient is generally selected to provide for

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the desired bulk, consistency, etc., when combined with components of a given pharmaceutical composition, in view of the intended administration mode. Typical pharmaceutical excipients include, but are not limited to binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc.).

The humanized antibody or the chimeric protein comprising same may be formulated for administration with a pharmaceutically-acceptable excipient, in unit dosage form or as a pharmaceutical composition. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such compositions to subjects. Although intravenous administration is preferred, any appropriate route of administration may be employed, for example, oral, parenteral, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intrathecal, epidural, intracisternal, intraperitoneal, intranasal, or aerosol administration. Therapeutic formulations may be in the form of liquid solutions or suspension. Methods well known in the art for making formulations are found in, for example, Remington: The Science and Practice of Pharmacy, (19th ed.) ed. A.R. Gennaro AR., 1995, Mack Publishing Company, Easton, PA.

In addition, in some embodiments, the humanized antibody or the chimeric protein can be administered at a pharmaceutically effective amount. The term "pharmaceutically effective amount" or "therapeutically effective amount" refers to an amount (dose) effective in treating a subject afflicted by or suspected to be afflicted by an thrombotic, metastatic or inflammatory condition or disorder. It is also to be understood herein that a "pharmaceutically effective amount" may be interpreted as an amount giving a desired therapeutic effect, either taken in one dose or in any dosage or route, taken alone or in combination with other therapeutic agents.

A therapeutically effective amount or dosage of the humanized antibody or the chimeric protein comprising same disclosed herein or a pharmaceutical composition, may range from about 0.001 to 30 mg/kg body weight, with other ranges of the invention including about 0.01 to 25 mg/kg body weight, about 0.025 to 10 mg/kg body weight, about 0.3 to 20 mg/kg body weight, about 0.1 to 20 mg/kg body weight, about 1 to 10 mg/kg body weight, 2 to 9 mg/kg body weight, 3 to 8 mg/kg body weight, 4 to 7 mg/kg body weight, 5 to 6 mg/kg body weight,

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and 20 to 50 mg/kg body weight. In other embodiments, a therapeutically effective amount or dosage may range from about 0.001 to 50 mg total, with other ranges of the invention including about 0.01 to 10 mg, about 0.3 to 3 mg, about 3 to 10 mg, about 6 mg, about 9 mg, about 10 to 20 mg, about 20-30 mg, about 30 to 40 mg, and about 40 to 50 mg. In an embodiment, the chimera is administered to a dosage between about 40-80 mg/kg (e.g. 60 mg/kg).

EXAMPLE I - HUMANIZATION OF MURINE NIT-B1 ANTIBODIES

The variable domains of murine NIT-A1 and NIT-B1 antibodies (described in US Patent Serial Number 8,323,652 and respectively deposited at the International Depositary Authority of Canada on October 7, 2008 under Accession Numbers 071008-01 (NIT A1 clone), 071008-02 (NIT B1 clone)) were sequenced. The murine NIT-A1 antibody has a heavy chain of SEQ ID NO: 1 (including CDR1 of SEQ ID NO: 3, CDR2 of SEQ ID NO: 4 and CDR3 of SEQ ID NO: 5) and a light chain of SEQ ID NO: 11 (including CDR1 of SEQ ID NO: 13, CDR2 of SEQ ID NO: 14 and CDR3 of SEQ ID NO: 15). The murine NIT-B1 antibody has a heavy chain of SEQ ID NO: 6 (including CDR1 of SEQ ID NO: 8, CDR2 of SEQ ID NO: 9 and CDR3 of SEQ ID NO: 10) and a light chain of SEQ ID NO: 16 (including CDR1 of SEQ ID NO: 18, CDR2 of SEQ ID NO: 19 and CDR3 of SEQ ID NO: 20).

The murine NIT-B1 antibody was further developed as a chimeric C100-Fab by fusing the NIT-B1 heavy chain variable domain (SEQ ID NO: 6) with a human IgG1 constant region CH1 (SEQ ID NO: 26; <https://www.uniprot.org/uniprot/P01857>); as well as fusing the NIT-B1 light chain variable domain (SEQ ID NO: 16) with a human Ig kappa light chain constant region (SEQ ID NO: 33; <http://www.uniprot.org/uniprot/P01834>).

EXAMPLE II – CHARACTERIZATION OF HUMANIZED ANTI-GPIBALPHA ANTIBODIES

Using CDR grafting method (Safdari *et al.*, 2013), four human heavy (VH1 of SEQ ID NO: 35, VH2 of SEQ ID NO: 42, VH3 of SEQ ID NO: 49, VH4 of SEQ ID NO: 56) and four human light (VL1 of SEQ ID NO: 63, VL2 of SEQ ID NO: 70, VL3 of SEQ ID NO: 77, VL4 of SEQ ID NO: 84) chains were synthesized based on homology of the frame works to human sequences in the NCBI database with the annotated CDRs.

Briefly, the variable domain sequences of parental antibody were searched in the database of human germline using NCBI Ig-Blast (<http://www.ncbi.nlm.nih.gov/projects/igblast/>). Four diverse human acceptors (i.e. human variable domains with high homology to the parental antibody) for each heavy chain and light chain were chosen. The CDRs of human acceptors

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were replaced with their mouse counterparts, resulting in the humanized variable domain sequences.

Humanized single-chain variable fragment (scFv) and associated chimeric protein. A scFv including both VH1 and VL2 was prepared. It included a (GGGGS (SEQ ID NO: 92)) x 4 linker between VH1 and VL2 (orientation as VH1-(G₄S)₄-VL2). For facilitating the purification, the scFv had a 6 x His-tag and a TEV cleavage site ENLYFQG prior to VH1. The tag was however removed using the TEV protease prior to the different testing. The scFv was also included in a chimeric protein (scFv-HSA) with the human serum albumin (HSA). In those instances, the scFv-HSA included an additional linker GGGGS (SEQ ID NO: 92) ahead of HSA. The chimeric scFv-HSA was produced in a stable form and did not form aggregates during its production or purification.

Size. The DNA sequences encoding humanized heavy and light chains were synthesized and inserted into pTT5 vector to construct expression plasmids of Fabs. Sixteen humanized Fabs were transiently expressed in HEK 293 or CHO 3E7 cell culture, and then the cells were spun down. The supernatants were filtered and evaluated with SDS-PAGE and Western-blot analysis. The humanized Fabs have molecular weight of approximately 47 kDa under non-reducing conditions (Figures 1 and 2).

The supernatants of eight selected humanized Fabs (see table 1) were then purified by Capture select™ Kappa XL Affinity Matrix resin. The purified humanized Fabs were buffer-exchanged into PBS using a PD-10 desalting column. The concentration and purity of the purified protein were determined by OD₂₈₀ and SDS-PAGE (about 2 µg of protein was loaded in each lane), respectively. As shown on Figure 3, the purified humanized Fabs migrated as ~47 kDa band in SDS-PAGE under non-reducing condition, ~ 24 kDa and ~ 23 kDa bands under reducing condition.

Table 1. Description of the humanized Fabs

Name	Heavy chain	Light chain
H001	VH1 (SEQ ID NO: 35)	VL2 (SEQ ID NO: 70)
H002	VH1 (SEQ ID NO: 35)	VL3 (SEQ ID NO: 77)
H003	VH2 (SEQ ID NO: 42)	VL1 (SEQ ID NO: 63)
H004	VH3 (SEQ ID NO: 49)	VL2 (SEQ ID NO: 70)
H005	VH3 (SEQ ID NO: 49)	VL3 (SEQ ID NO: 77)
H006	VH4 (SEQ ID NO: 56)	VL1 (SEQ ID NO: 63)
H007	VH4 (SEQ ID NO: 56)	VL2 (SEQ ID NO: 70)
H008	VH4 (SEQ ID NO: 56)	VL3 (SEQ ID NO: 77)

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Specificity. Human and mouse platelet-rich plasma (PRP) was prepared by centrifugation at 300g for 7 min, and was washed by transferring 200 μ L PRP into 10 mL PBS followed with 800g centrifugation for 10 min. The supernatant was then removed and platelets were re-suspended in 200 μ L PBS. Washed platelets (10 μ L) were incubated with the different
 5 antibodies (2.5-5 μ g/mL) in a 200 μ L system at room temperature for 30 min, and detected by FITC labeled anti-human-Fab antibody. These eight selected humanized Fabs H001-H008, the scFv and the chimeric protein all bound to both human and wild-type mouse platelets, but not GPIb α deficient mouse platelets, indicating the specificity of the humanized Fabs to platelet GPIb α (Figures 4 and 19).

10 Platelets (2×10^5) from mouse, dog, human, rat and rabbit PRP were transferred into 200 μ L PBS containing a serial concentration of the Fabs H001 or H002 at 50, 16.7, 5.6, 1.8, 0.6, 0.2 nM (for mouse and dog), at 200, 67, 22, 7.4, 2.5, 0.8, 0.3, 0.1 nM (for human and rat) and at 500, 100, 20, 4, 0.8 nM (for rabbit). After a 30 min incubation, a detection antibody (FITC-labeled anti-human Kappa chain, 1:200) was added and incubated in darkness for 15 min.
 15 Flow cytometry was performed using the BD LSR Fortessa™ X-20. H001 and H002 also bound to rat, dog and rabbit platelets (Figure 5).

Humanized Fabs with (purified H001/H002, 5 μ g/mL) and without (non-purified H001/H002, 5 μ g/mL) the second purification by SEC-FPLC chromatography were incubated with Cynomolgus monkey washed platelets (2×10^6) for 30 min. A FITC-labeled anti-human
 20 Kappa chain secondary antibody (Ab) was then incubated for 30 minutes. Humanized Fabs binding to monkey platelets were detected by flow cytometry assay. Figure 6 shows that Fab H001 and H002 antibodies bound to monkey platelets.

The affinity between H001 and recombinant GPIb α was measured in a surface plasmon resonance (SPR) assay. For preparation of SPR biosensors, SPR bare gold coated
 25 biosensors (Biosensing Instrument Inc., AZ, USA) were cleaned in a solution of 0.5M sodium borohydride dissolved in 1:1 anhydrous ethanol:ddH₂O for 2 hours. The biosensors were rinsed with copious amounts of anhydrous ethanol followed by 16 hour incubation in a solution of 1 mM mercaptopropionic acid in dimethylformamide. Following the incubation, SPR biosensors were rinsed with copious amounts of dimethylformamide followed by
 30 anhydrous ethanol and finally ddH₂O. The biosensors were then incubated in 40 mM 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide and 20 mM N-Hydroxysuccinimide dissolved in ddH₂O for 1 hour. Sensors were then rinsed with copious amounts of ddH₂O and then incubated with 100 mM N α ,N α -Bis(carboxymethyl)-L-lysine hydrate for 4 hour. Following the incubation, the biosensing surfaces were rinsed with ddH₂O. The final step before SPR

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experiments was to expose the functionalized biosensing surfaces with 100 mM NiCl_2 for 1 hour.

The SPR experiments were performed on a Biosensing Instrument 4000 SPR. The functionalized biosensors were loaded onto the instrument and the biosensor was
5 equilibrated with running buffer (10 mM tris(hydroxymethyl)aminomethane, 140 mM NaCl, 20 mM Imidazole pH = 7.4) at a flow rate of 40 $\mu\text{L}/\text{min}$. For binding measurements 25 μL of 500 nM hexa-histidine tagged GPIb α was injected over the biosensing surface immobilizing the GPIb α to the SPR sensor surface. The baseline was allowed to equilibrate followed by injection of 25 μL of the desired concentration of ligand (either Fab H001 or control).
10 Biosensing surfaces were regenerated between ligand injections by injection of 100 μL of 500 mM imidazole in running buffer, followed by 100 μL of 100 mM NiCl_2 . The resulting data was analyzed using the instrument included SPR analysis software.

GPIb α immobilized SPR biosensors were exposed to 500, 100, 50 and 10 nM of Fab H001 antibody and 100 nM control antibody (PSI E1, an antibody against GPIIb/IIIa). The control
15 did not bind the immobilized GPIb α and fully dissociated from the biosensor prior to the end of injection (data not shown). In contrast the Fab H001 antibody clearly bound GPIb α producing clear SPR shifts with only partial dissociation after the end of the injection (Fig. 7A). The SPR shifts were fit to the kinetics binding model resulting in an on rate (k_a) of $2.61 \times 10^7 \text{ s}^{-1}$ an off rate (k_d) = $1.1 \times 10^{-1} \text{ s}^{-1}$ and a dissociation constant (K_d) of 4.4 nM (Fig. 7B). To
20 confirm the dissociation constant, the magnitude of the SPR shifts were plotted against the Fab H001 antibody concentration and fit to a one site binding model producing a fit R^2 of 0.9929 and a dissociation constant (K_d) of $8.0 \pm 2.1 \text{ nM}$. The data clearly demonstrates that the Fab H001 antibody bound recombinant GPIb α tightly with a low nanomolar dissociation constant and a fast binding on rate.

25 *In vitro inhibition of agonists-induced platelet aggregation.* To evaluate whether the humanized Fabs can induce aberrant platelet activation, and their roles in platelet aggregation, *in vitro* platelet aggregation assays were performed. Human PRP from healthy volunteers and patients with peripheral vascular disease was prepared from sodium-citrate anti-coagulated whole blood by centrifugation at 300g for 7 min. Platelet aggregation in PRP
30 was induced by the addition of 5 $\mu\text{g}/\text{mL}$ humanized Fab clones and monitored by a computerized Chrono-log aggregometer (Chrono-Log Corporation, USA). Platelet aggregation in PRP was induced by ristocetin (1 mg/mL), and in gel-filtered platelets was induced by thrombin (0.05 U/mL) with or without the humanized Fabs using a computerized Chrono-log aggregometer (Chrono-Log Corporation, USA).

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The Fab H001, H002, H005 and H008 antibodies as well as the scFv antibody did not induce platelet activation. Moreover, H001 and H002 significantly inhibited ristocetin-induced human platelet aggregation in PRP, and low-dose thrombin-induced platelet aggregation in gel-filtered platelets (Figures 8, 9, 20 and 21).

5 *Inhibition of thrombus formation at low and high shear rate.* To measure platelet adhesion, aggregation, and thrombus formation at different shear rates, heparinized-whole blood from 30 healthy volunteers was perfused over a type I collagen-coated surface using an *ex vivo* perfusion chamber system, under a real-time fluorescence microscope. Briefly, rectangular microcapillary tubes (ibidi channel slides, ibidi GmbH) were coated with Horm collagen (100
10 $\mu\text{g/mL}$, overnight, 4°C ; Nycomed Linz, Austria). Anti-coagulated (heparin 15 U/mL) whole blood from healthy donors was fluorescently-labeled with DiOC₆ (1 μM , 10 min, 37°C ; Sigma). Then, control or humanized Fabs or scFv-HSA treated whole blood was perfused over the collagen-coated surface at shear rates of 300 s^{-1} , $1\ 200\text{ s}^{-1}$ and $1\ 800\text{ s}^{-1}$ for 3 min using a syringe pump (Harvard Apparatus, USA). Platelet accumulation and thrombus
15 formation were recorded in real-time under a Zeiss Axiovert 135-inverted florescent microscope (60 $\times$ /0.90 NA water objective). Quantitative dynamics of platelet fluorescence intensity were acquired using SlideBook software.

The humanized Fabs H001 and H002 markedly inhibited thrombus formation at both 300 s^{-1} and $1\ 800\text{ s}^{-1}$ wall shear rates (although preferably at $1\ 800\text{ s}^{-1}$ wall shear rates),
20 corresponding to blood flow in venules/large arteries and arterioles, respectively (Figure 10). The chimeric protein markedly inhibit thrombus formation at $1\ 200\text{ s}^{-1}$ wall shear rates (Figure 22). These *ex vivo* results suggest that humanized C100-Fab, scFv and chimeric proteins are significant inhibitors of thrombosis under both low shear and high shear conditions.

In vivo inhibition of thrombus growth and vessel occlusion. To examine whether the
25 humanized Fabs affects thrombus growth *in vivo*, two complementary intravital microscopy thrombosis models and a large artery thrombosis model were utilized.

Thrombus formation in mesenteric arterioles was monitored in 3- to 4-week-old C57BL/6 wild-type mice. Mice were injected with donor-matched fluorescently labeled platelets and visualized under a Zeiss Axiovert 135-inverted fluorescent microscope (Zeiss, Germany).
30 Briefly, blood was collected into an acid citrate dextrose solution (ACD; as anti-coagulant) from the donor-matched mice. Gel-filtered platelets were prepared and labeled with Calcein AM (1 mg/mL, Invitrogen, Canada) at room temperature for 20 min. Platelets were then injected into the experimental mice via the tail vein with control saline buffer, with Fab H001 or with H002 (2.5 or 5 $\mu\text{g}/\text{mouse}$). Mice were then anesthetized and the mesentery was

externalized. A single mesenteric arteriole of 100-120 µm diameter was chosen and injury was induced by topical application of 30 µL of 250 mM ferric chloride. The time to complete vessel occlusion was recorded. Images of thrombus formation and dissolution were visualized with a fluorescence microscope. As shown in Figure 11 and table 2, thrombus growth and vessel occlusion induced by FeCl₃ injury were significantly inhibited by injection of the humanized Fabs H001 and H002 antibodies (when compared to a control saline injection).

Table 2. Number of mice that did not occlude in function of treatment received.

Control	0
H002 – 5 µg	1 in 4
H001 – 5 µg	3 in 4
H001 – 2.5 µg	0

For the laser-induced cremaster arteriole thrombosis model, C57B/6 wild-type mice (male, 6-8 weeks old) were anesthetized and a tracheal tube was inserted to facilitate breathing. The cremaster muscle was prepared under a dissecting microscope and superfused throughout the experiment with preheated bicarbonate-buffered saline. Platelet antibody, control (saline buffer), the humanized and monovalent H001 and H002 antibodies (5 or 10 µg/mouse) were administered where indicated by a jugular vein cannula. Platelets were labeled by the rat anti-mouse CD41 antibody (Leo.A1; EMFRET Analytics, Germany; 0.1 µg/g) injection. Multiple independent upstream injuries were induced on a cremaster arteriole using an Olympus BX51WI microscope with a pulsed nitrogen dye laser. The dynamic accumulation of fluorescently labeled platelets within the growing thrombus was captured and analyzed using Slidebook software. In this cremaster arteriole intravital microscopy thrombosis model (which does not involve oxidative stress as it induces mild vascular injury with a laser) the thrombus growth was almost completely abolished after intravenous infusion of the humanized and monovalent H001 and H002 antibodies (Figure 12). These findings indicate that the humanized Fabs can inhibit thrombus growth and promote thrombus dissolution, and therefore have great potential to be developed as novel anti-thrombotic agents.

Mouse blood was drawn before and after the intravital microscopy thrombosis model experiments and the platelets were isolated and characterized using flow cytometry and FITC-labeled mouse anti-human CD62P antibody and Annexin V- Alexa Fluor® 647. It is shown in Figure 13 that the humanized and monovalent H001 antibody did not induce platelet aberrant activation *in vivo* as it did not result in platelet P-selectin expression or

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phosphatidyl serine (PS) exposure. Similar results were obtained with the humanized Fab H002 antibody (data not shown).

In a ferric chloride-induced large carotid artery thrombosis model, C57BL/6J wild-type mice (both genders, >8-week old, 25–30g) were anesthetized and intravenously injected with Fab H001 or H002 antibody (5, 10 or 20 µg/mouse) or an equal volume (200 µL) of PBS 5 minutes before inducing arterial injury. The left common carotid artery was dissected and held with a miniature Doppler flow probe (TS420 transit-time perivascular flowmeter, Transonic Systems Inc., USA). The baseline blood flow rate was measured for 30 seconds. Carotid artery injury was then induced with a strip of Whatman filter paper saturated with 7.5% ferric chloride for 3 minutes. Blood flow was monitored until complete vessel occlusion was observed. The Fabs H001 and H002 antibodies significantly reduced thrombus growth and prevented stable vessel occlusion (Figure 14).

In vivo decrease of the infarct size of ischemic brain without increasing the risk of intracerebral hemorrhage. To investigate the therapeutic potential of the antibodies in ischemic stroke, a cerebral ischemia and reperfusion injury model (transient middle cerebral artery occlusion (tMCAO)) was conducted. Male mice (25 g) were anesthetized with inhaled isofluorane. A midline neck incision was made and the soft tissues were pulled apart. The left common carotid artery (LCCA) was carefully dissected free from the surrounding nerves (without harming the vagal nerve) and a ligature was made using 5.0 string. The left external carotid artery (LECA) was then separated and a second knot was made. Next, the left internal carotid artery (LICA) was isolated and a knot was prepared with a 6.0 filament. After obtaining good view of the left internal carotid artery (LICA) and the left pterygopalatine artery (LPA), both arteries were clipped using a microvascular clip. A small hole was cut in the LCCA before it bifurcated to the LECA and the LICA. A standardized silicon rubber-coated 6.0 nylon monofilament (6021; Doccol Corp, Redlands, CA) was introduced into the LICA, until it stopped at the clip. The clipped arteries were opened while the filament was inserted into the LICA to occlude the origin of the LMCA in the circle of Willis. The third knot on the LICA was closed to fix the filament in position. After 1 hour, the third knot was opened and the filament was withdrawn. The antibodies were administered intravenously immediately after the filament was inserted; or after 1 hour when the filament was withdrawn.

To measure cerebral infarct volumes, mice were euthanized 24 hours after induction of tMCAO. Multiple 2 mm-thick coronal brain sections cut from the whole brain were stained with 2% 2,3,5-triphenyl-tetrazolium chloride (TTC, Sigma-Aldrich, St Louis, MO) to visualize cerebral infarctions. The presence of cerebral hemorrhages was macroscopically assessed. To measure the neurological function twenty-four hours after induction of tMCAO, mice were

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subjected to the modified Bederson test and the grip test to assess global neurological and motor function respectively. Results showed that blocking of GPIIb/IIIa by the humanized Fabs H001 and H002 antibodies and scFv-HSA significantly reduced infarct size in the brain and improved functional outcome after tMCAO without increasing the risk of intracerebral hemorrhage (Figure 15).

In vivo protection of TTP. To test the therapeutic effect of the antibodies in TTP, an ionophore-provoked ultra-large VWF (ULVWF)-mediated microvascular thrombosis model was used. ADAMTS13^{-/-} mice were anesthetized and intravenously injected with fluorescently labeled platelets purified from the donor mice of the same genotype, and ionophore-provoked microvascular thrombosis in mesenteric venules was monitored in real-time under intravital microscopy. For platelet preparation, mice (6–8 weeks old) were anesthetized by intraperitoneal injection of ketamine/xylazine (100 mg/kg and 10 mg/kg body weight, respectively), and whole blood was collected from the retro-orbital plexus using heparin-coated glass capillary tubes. Blood was collected into a tube containing citrate-dextrose solution (38 mmol/L citric acid, 75 mmol/L trisodium citrate, 100 mmol/L dextrose). Platelet-rich plasma was obtained by centrifugation of whole blood at 300g for 7 minutes. Gel-filtered platelets were then isolated from the platelet-rich plasma using a sepharose 2B column in PIPES buffer (PIPES 5 mmol/L, NaCl 1.37 mmol/L, KCl 4 mmol/L, and glucose 0.1%, pH 7.0). Platelet counts were confirmed using a Hemovet (HV950, Drew Scientific). Fluorescent labeling of gel-filtered platelets was achieved by incubating platelets with calcein-acetoxymethyl ester (1 µg/mL) for 15 minutes at room temperature. The efficacy of the fluorescent labeling of platelets was confirmed under fluorescent microscope before being used for *in vivo* imaging.

For intravital microscopy imaging, 4-week-old mice were anesthetized and injected with fluorescently labeled platelets (1.25×10^6 platelets/g from mice of the same genotype). Mesenteric vessels were surgically prepared and monitored under an inverted fluorescent microscope (Zeiss Axio Observer Z1 Advanced Marianas Microscope) using a 25× oil objective lens (Zeiss). An ≈ 2.5 mm section of the mesenteric venule (100 to 150 µm in diameter) was topically treated with 10 µL of 10 µmol/L of calcium ionophore to induce Weibel-Palade body secretion of ULVWF from the endothelium, resulting in immediate adhesion of fluorescently labeled platelets and formation of platelet thrombi anchored to the vessel wall. For each mouse, the process of thrombosis and the resolution of thrombi were monitored and recorded for 20 minutes in addition to prerecording. The Fab H001 or the scFv-HSA chimeric protein was administered via tail vein catheter 10 minutes before (prophylactic) the onset of thrombosis by calcium ionophore stimulation in mesenteric

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microvasculature in ADAMTS13^{-/-}. The dynamics of platelet accumulation in selected vessel segments was quantitatively analyzed by (1) number of emboli (platelet thrombi larger than 20 µm in diameter), and (2) time to restore normal blood flow: defined as the time required for platelet fluorescence to return to near baseline after the topical application.

5 As shown in the Figure 16, no platelet adhesion to the mesenteric vessel wall was detected under intravital microscopy prior to the application of ionophore in all ADAMTS13^{-/-} mice that treated with saline (control), the Fab H001 antibody, or the scFv-HSA chimeric protein. Immediately after the topical application of calcium ionophore to the mesenteric vessel in control ADAMTS13^{-/-} mice, platelet adhesion was observed onto mesenteric venules,
10 presented as single platelet strings formation attached to endothelial in the direction of blood flow. Within a minute, multiple large thrombi (>20 µm in diameter) formed and some grew up to 50% of the diameter of the ionophore-treated vessels. Platelet string and thrombi were visibly very loose and easily detach from vessel wall and emboli to downstream. Platelet adhesion to vessel wall and embolic thrombosis in control mice continued up to 10 minutes
15 or more but decreased over the time and ultimately restored the normal blood flow in effected section of the vessel. The thrombotic response in ADAMTS13^{-/-} mice was dramatically inhibited by prophylactic treatment of both the Fab H001 antibody or the scFv-HSA chimeric protein (Figure 16). Platelet adhesion onto vessel wall and formation of large thrombi were strongly inhibited by both the Fab H001 antibody or the scFv-HSA chimeric protein treatment
20 (Figure 16). The numbers of large emboli thrombi were significantly less and the time to restore normal blood flow in mesenteric venules as shorter in both in the Fab H001 antibody or the scFv-HSA chimeric protein treated group when compared to control group (Figure 16). These results demonstrated that prophylactic treatment of ADAMTS13^{-/-} mice with the Fab H001 antibody or the scFv-HSA chimeric protein effectively inhibited ionophore-provoked
25 VWF-mediated microvascular thrombosis, mimicking platelet accumulation on newly released endothelial-bound ULVWF in TTP.

Inhibition of thrombocytopenia. C57BL/6 mice were injected intravenously with intravenous immunoglobulins (IVIg), the humanized Fab H001 or H002 antibody (10 µg/mouse, n=3 each group), or the NIT-B1 antibody (5 µg/mouse, n=2). A series of blood samples at different time
30 points (0, 30 min, 1, 2, 4, 8 hours, 1, 2, 3, 4, 5, 6 and 7 days) were collected from mice medial saphenous vein. At each time point, 10 µL blood sample were collected and added into 240 µL 1% PBS-EDTA (pH 7.4) to prevent clotting. For platelets counting, 50 µL blood sample (in PBS-EDTA) was transferred into 10 mL diluent (Isoton II, Coulter Corporation) and the platelets count was determined using a Coulter counter (Beckman Z2, Coulter Corporation). As shown in Figure 17, the humanized and monovalent H001 or H002
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antibodies did not induce a significant loss in platelet count in the first 24 h following their administration which is in clear contrast to the NIT-B1 antibody.

Bleeding time. BALB/c mice were injected intravenously with PBS (n=4), the Fab H001 or H002 antibody (5-10ug/ mouse, n=3) 120 mins before injury. Mice were anesthetized by 2.5% avertine (18 mL/kg body weight, i.p.), and maintained on a 37°C heating pad. The tip of the tail (2 mm) was cut off with a sharp scalpel and a tissue paper was used to tap wound every 15 seconds. Bleeding time was recorded as the time to cessation of blood flow (bleeding stopped for >10 s). The assay was terminated after 15 minutes if the tail was still bleeding. As shown in Figure 18, the administration of the H001 or the H002 antibody did not prolong bleeding time.

REFERENCES

- Yaghoub Safdari , Safar Farajnia , Mohammad Asgharzadeh & Masoumeh Khalili (2013) Antibody humanization methods – a review and update, Biotechnology and Genetic Engineering Reviews, 29:2, 175-186
- While the invention has been described in connection with specific embodiments thereof, it will be understood that the scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

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WHAT IS CLAIMED IS:

1. A humanized antibody specifically recognizing platelet glycoprotein I(b) α (GPIb α), wherein the humanized antibody lacks a Fc moiety and:
 - is capable of preventing platelet activation, aggregation, and/or thrombus growth;
 - lacks the ability to activate platelets;
 - lacks the ability to induce thrombocytopenia; and/or
 - at a therapeutic dose, lacks the ability to prolong bleeding time.
2. The humanized antibody of claim 1 being capable of recognizing a human GPIb α , a mouse GPIb α , a dog GPIb α , a rat GPIb α , a rabbit GPIb α and/or a monkey GPIb α .
3. The humanized antibody of claim 1 or 2 being an antibody fragment.
4. The humanized antibody of claim 3 being a F(ab)₂ fragment.
5. The humanized antibody of claim 3, wherein the antibody is a monovalent antibody.
6. The humanized antibody of claim 5 being is a Fab antibody fragment.
7. The humanized antibody of claim 5 being a single chain variable fragment (scFv).
8. The humanized antibody of any one of claims 1 to 7 having a heavy chain.
9. The humanized antibody of claim 8, wherein the heavy chain comprises:
 - a first CDR having an amino acid sequence of GFTFSSFAMS (SEQ ID NO: 37), a variant thereof or a fragment thereof;
 - a second CDR having an amino acid sequence of SITSAGTPYYPDVSLG (SEQ ID NO: 38), a variant thereof or a fragment thereof; and/or
 - a third CDR having an amino acid sequence of SRGYEDYFDY (SEQ ID NO: 39), a variant thereof or a fragment thereof.
10. The humanized antibody of claim 8 or 9, wherein the heavy chain further comprises a CH1 region of a human IgG₁ antibody.
11. The humanized antibody of claim 10, wherein the CH1 region of the human IgG₁ antibody has the amino acid sequence of SEQ ID NO: 40, 47, 54 or 61, a variant thereof or a fragment thereof.
12. The humanized antibody of claim 11, wherein the heavy chain has the amino acid sequence of SEQ ID NO: 36, 43, 50 or 57, a variant thereof or a fragment thereof.

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13. The humanized antibody of any one of claims 1 to 12 having a light chain.
14. The humanized antibody of claim 13, wherein the light chain comprises:
 - a first CDR having an amino acid sequence of KSSQSLNLSRNQKNYLA (SEQ ID NO: 65), a variant thereof or a fragment thereof;
 - 5 – a second CDR having an amino acid sequence of FTSTRES (SEQ ID NO: 66), a variant thereof or a fragment thereof; and/or
 - a third CDR having an amino acid sequence of QQHYSSPWT (SEQ ID NO: 67), a variant thereof or a fragment thereof.
15. The humanized antibody of claim 13 or 14, wherein the light chain further comprises
10 a kappa chain C region of a human IgG₁ antibody.
16. The humanized antibody of claim 15, wherein the kappa chain C region has the amino acid sequence of SEQ ID NO: 68, 75, 82, or 89, a variant thereof or a fragment thereof.
17. The humanized antibody of claim 16, wherein the light chain has the amino acid
15 sequence of SEQ ID NO: 64, 71, 78 or 85, a variant thereof or a fragment thereof.
18. The humanized antibody of any one of claims 1 to 17 having:
 - the heavy chain of SEQ ID NO: 36, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 64, a variant thereof or a fragment thereof;
 - the heavy chain of SEQ ID NO: 36, a variant thereof or a fragment thereof and
20 the light chain of SEQ ID NO: 71, a variant thereof or a fragment thereof;
 - the heavy chain of SEQ ID NO: 36, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 78, a variant thereof or a fragment thereof;
 - the heavy chain of SEQ ID NO: 36, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 85, a variant thereof or a fragment thereof;
 - 25 – the heavy chain of SEQ ID NO: 43, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 64, a variant thereof or a fragment thereof;
 - the heavy chain of SEQ ID NO: 43, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 71, a variant thereof or a fragment thereof;
 - the heavy chain of SEQ ID NO: 43, a variant thereof or a fragment thereof and
30 the light chain of SEQ ID NO: 78, a variant thereof or a fragment thereof;

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- the heavy chain of SEQ ID NO: 43, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 85, a variant thereof or a fragment thereof;
 - the heavy chain of SEQ ID NO: 50, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 64, a variant thereof or a fragment thereof;
 - 5 – the heavy chain of SEQ ID NO: 50, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 71, a variant thereof or a fragment thereof;
 - the heavy chain of SEQ ID NO: 50, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 78, a variant thereof or a fragment thereof;
 - 10 – the heavy chain of SEQ ID NO: 50, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 85, a variant thereof or a fragment thereof;
 - the heavy chain of SEQ ID NO: 57, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 64, a variant thereof or a fragment thereof;
 - the heavy chain of SEQ ID NO: 57, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 71, a variant thereof or a fragment thereof;
 - 15 – the heavy chain of SEQ ID NO: 57, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 78, a variant thereof or a fragment thereof; or
 - the heavy chain of SEQ ID NO: 57, a variant thereof or a fragment thereof and the light chain of SEQ ID NO: 85, a variant thereof or a fragment thereof.
19. A chimeric protein comprising the humanized antibody of any one of claims 1 to 18
20 and a carrier protein.
20. A pharmaceutical composition comprising (i) the humanized antibody of any one of claims 1 to 18 or the chimeric protein of claim 19 and (ii) a pharmaceutical excipient.
21. A method of preventing or limiting the interaction between glycoprotein I(b) α (GPIb α) present on a platelet and a GPIb α ligand, the method comprising contacting the
25 humanized antibody of any one of claims 1 to 18, the chimeric protein of claim 19 or the pharmaceutical composition of claim 20 with the platelet.
22. The method of claim 21 for preventing or limiting platelet activation.
23. The method of claim 21 or 22, wherein the GPIb α ligand is von Willebrand factor (VWF) and/or thrombin.
- 30 24. The method of any one of claims 21 to 23, wherein the contacting occurs under low or high shear rates.

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25. The method of any one of claims 21 to 24, wherein the humanized antibody, the chimeric protein or the pharmaceutical composition is contacted with the platelet prior to, at the same time or after the GPIIb/IIIa ligand is contacted with the platelet.
- 5 26. The method of any one of claims 21 to 25 for preventing or limiting the interaction *in vivo* in a subject in need thereof.
27. The method of claim 26 for preventing the formation or the growth of a thrombus in the subject in need thereof.
28. The method of claim 26 for reducing the size of a thrombus or the number of thrombi in the subject in need thereof.
- 10 29. The method of any one of claims 26 to 28 further comprising determining the presence, the location and/or the size of the thrombus in the subject.
30. The method of any one of claims 26 to 28, wherein the subject is at risk of experiencing or has experienced a pathological thrombosis.
- 15 31. The method of any one of claims 26 to 28, wherein the subject is at risk of experiencing or has experienced an ischemic stroke, a thrombotic thrombocytopenic purpura, a myocardial infarct, an acute coronary syndrome, atherothrombosis, a peripheral vascular disease, deep vein thrombosis, sepsis, and/or a vascular inflammation.
- 20 32. The method of any one of claims 26 to 28 for reducing or limiting tumor metastasis in the subject in need thereof.
33. The method of claim 32 further comprising determining the presence, the location and/or the size of the tumor metastasis in the subject.

Figure 1

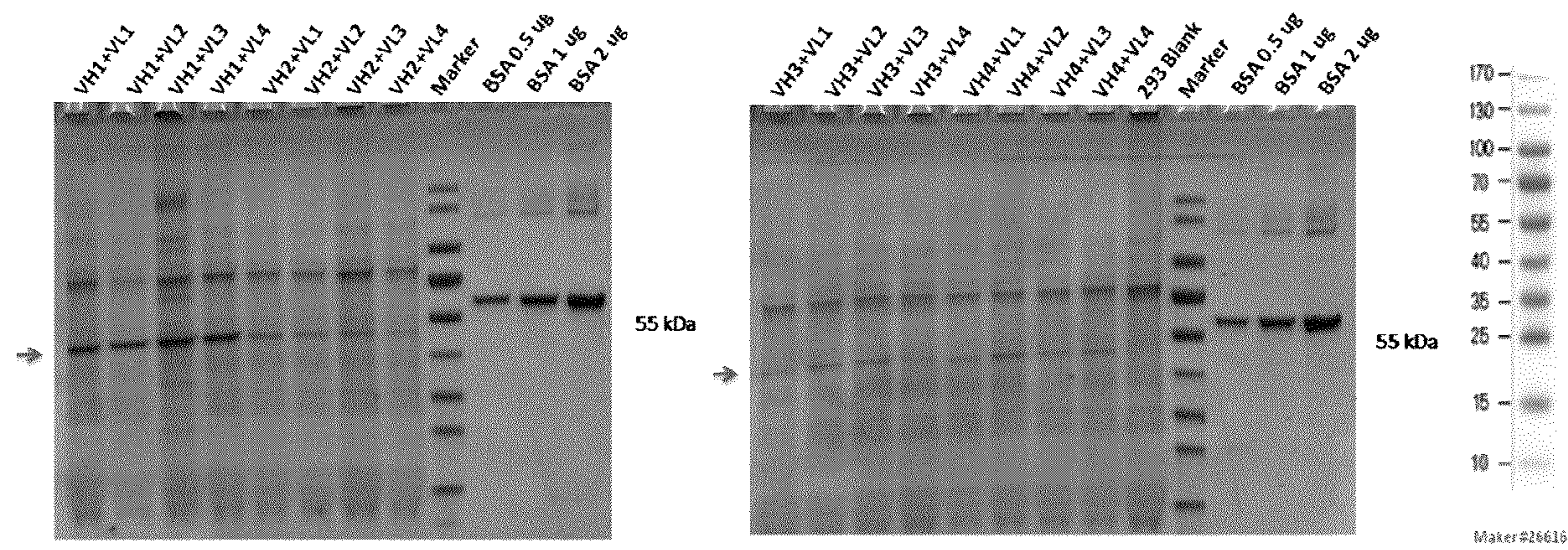


Figure 2

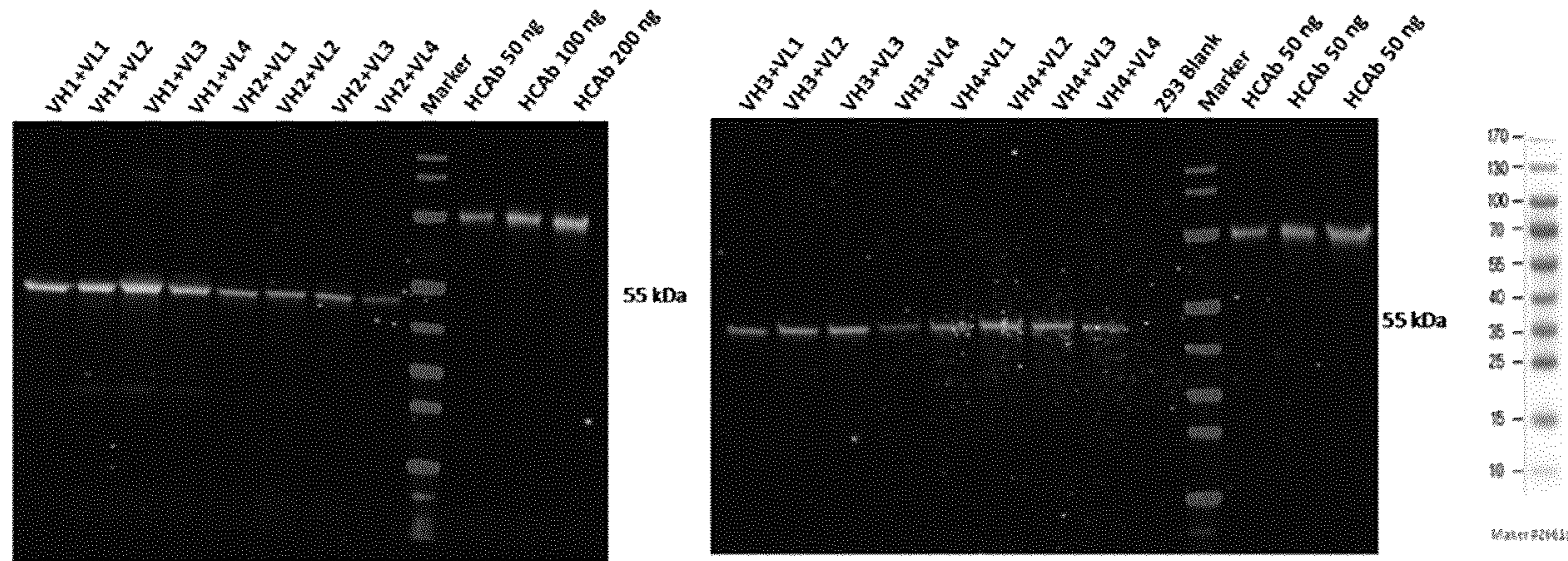


Figure 3

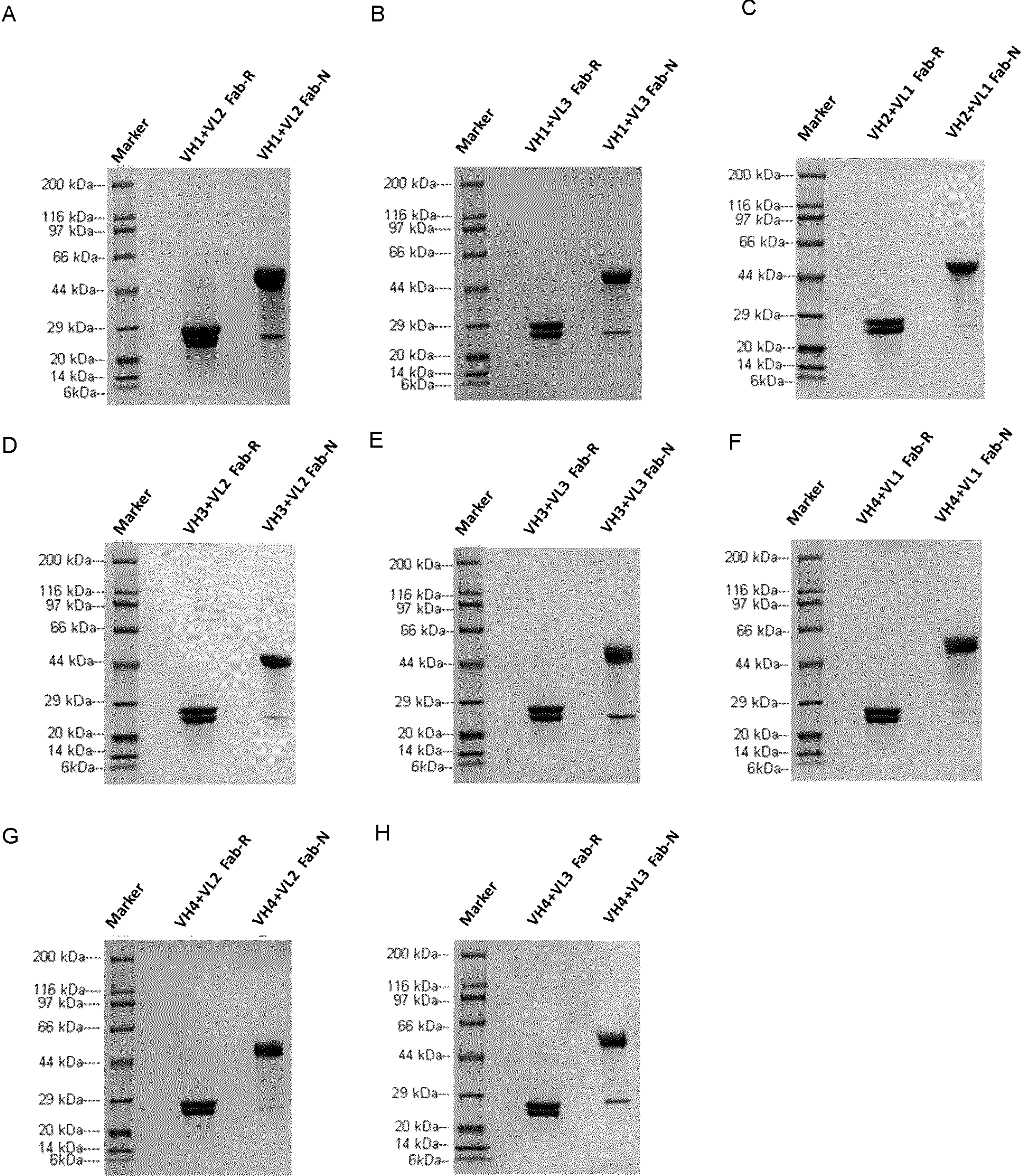


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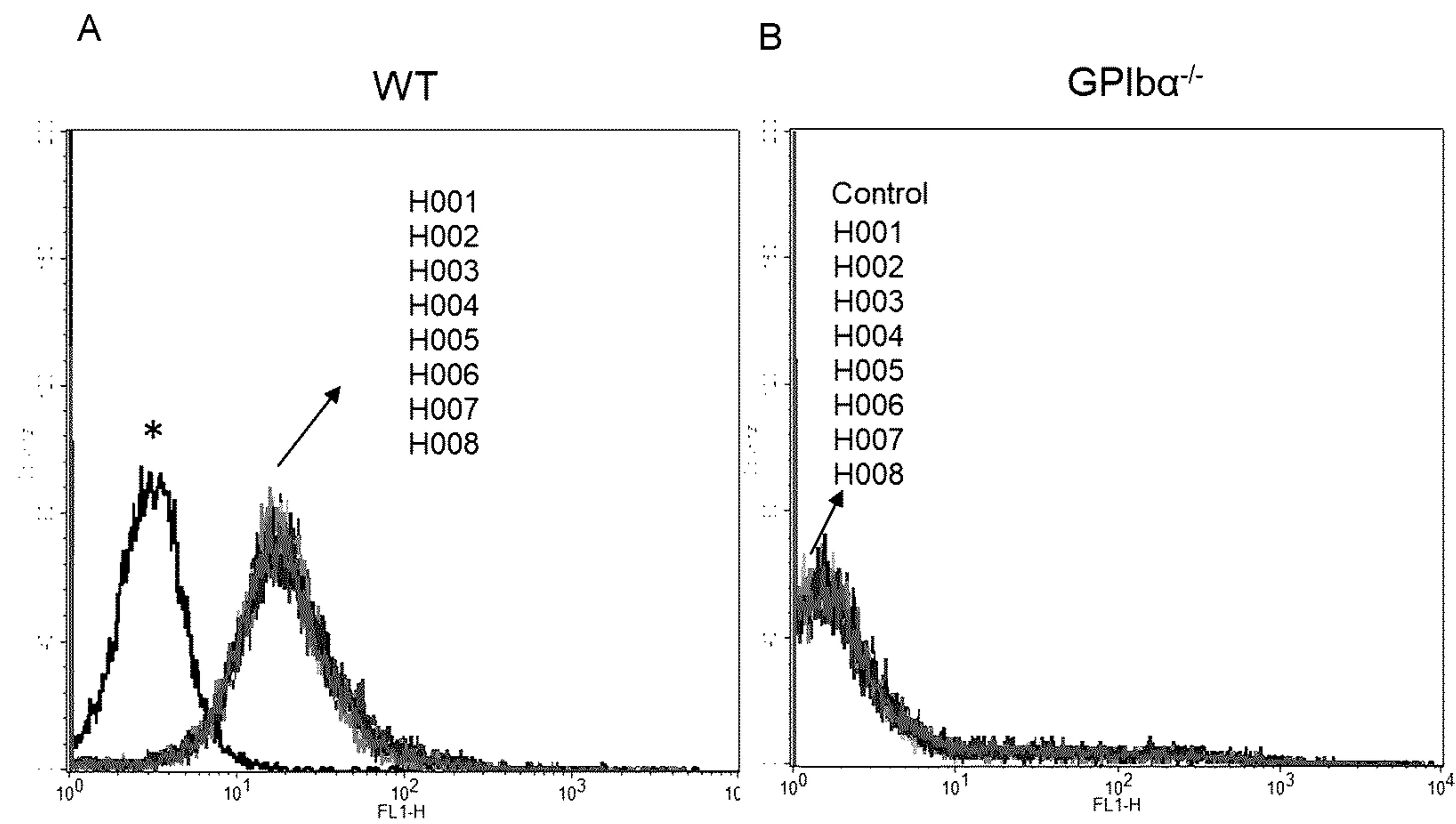


Figure 5

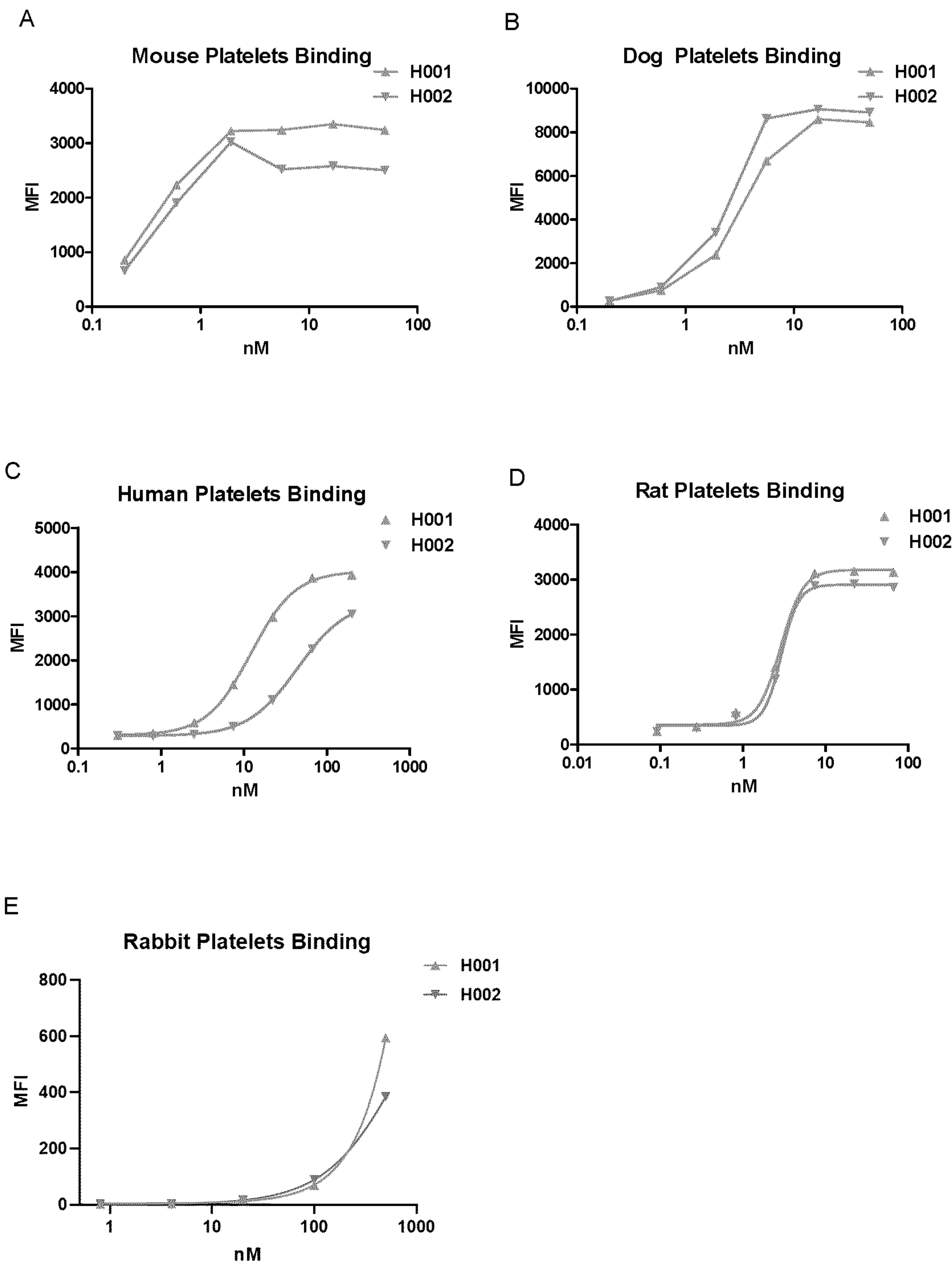


Figure 6

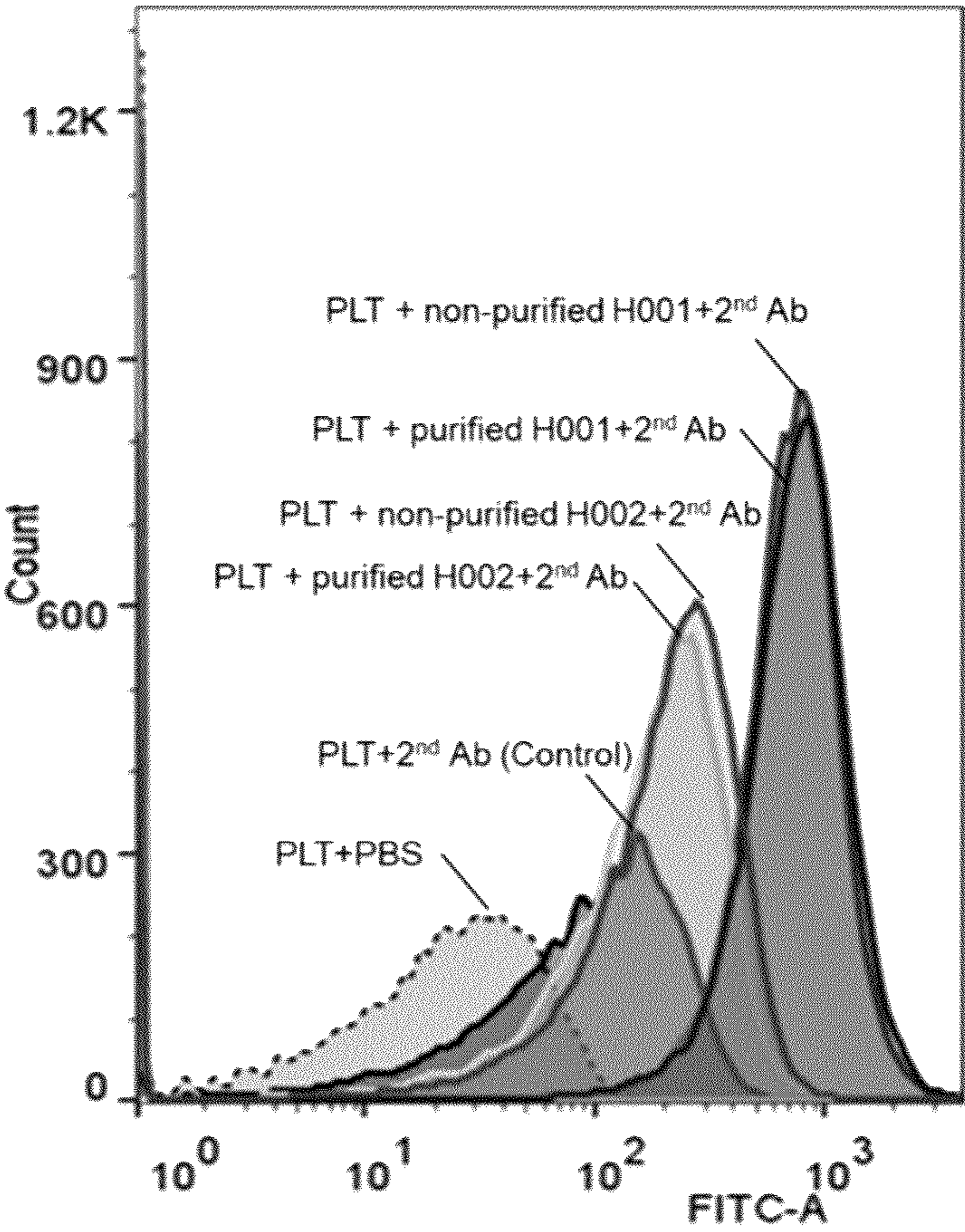
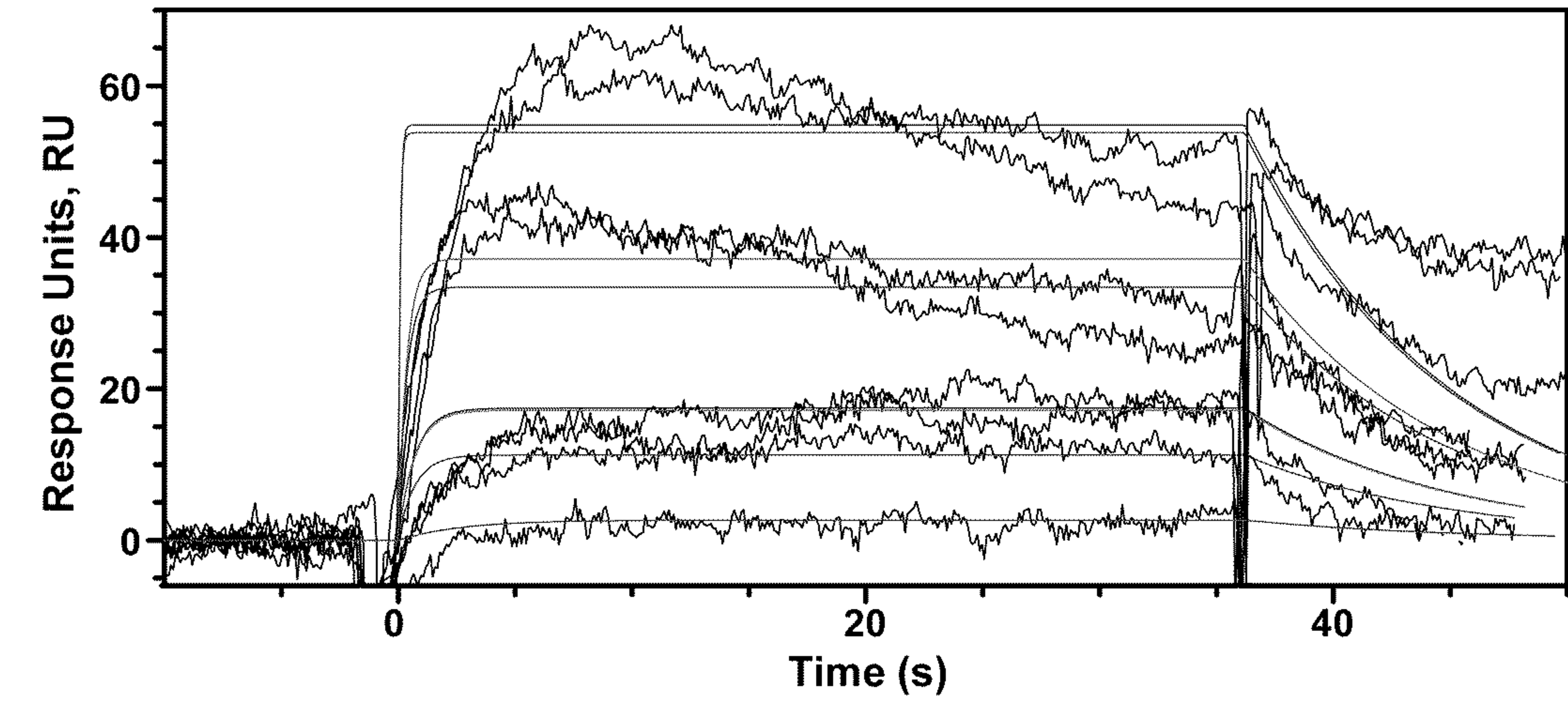


Figure 7

A



B

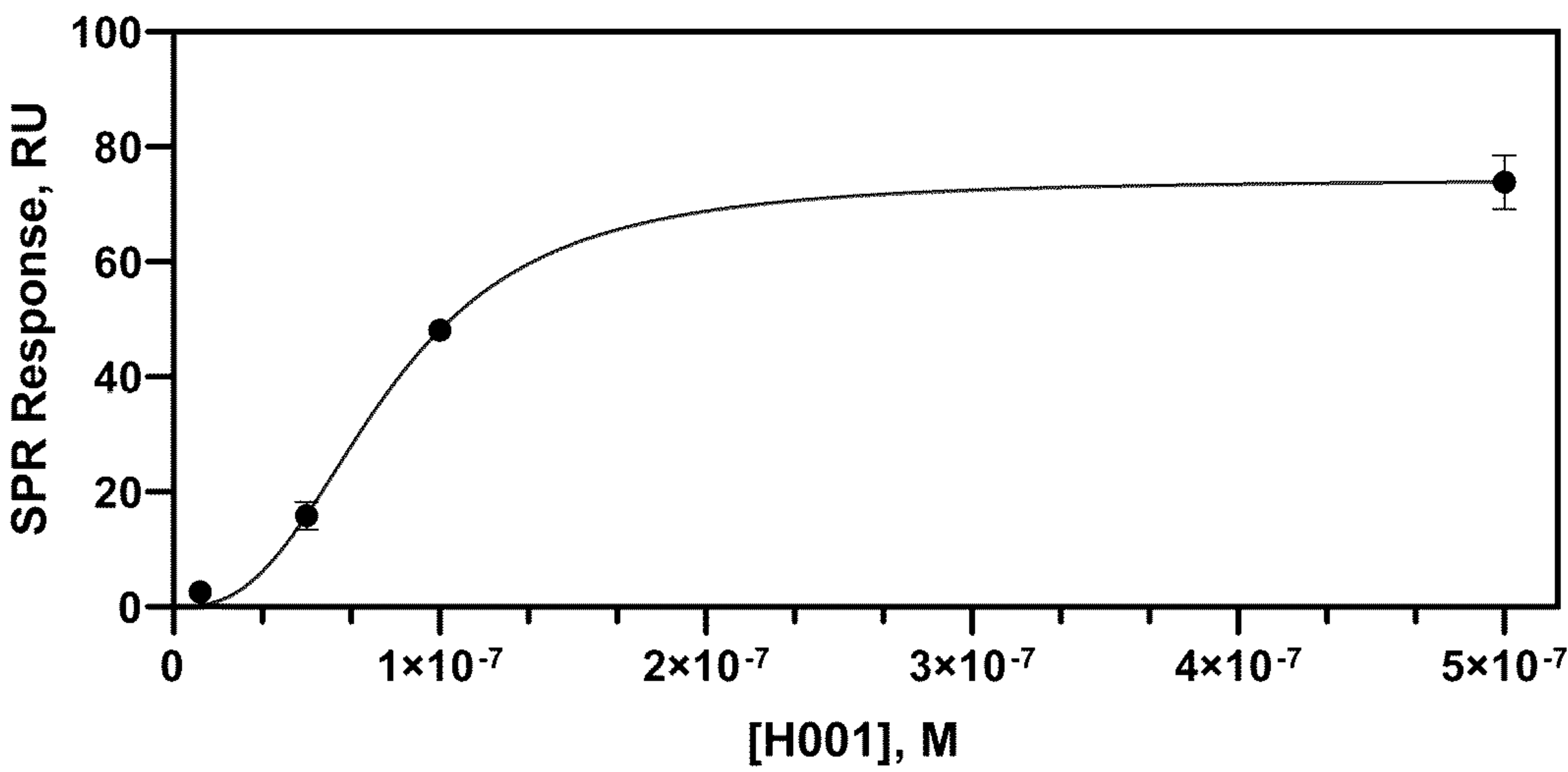
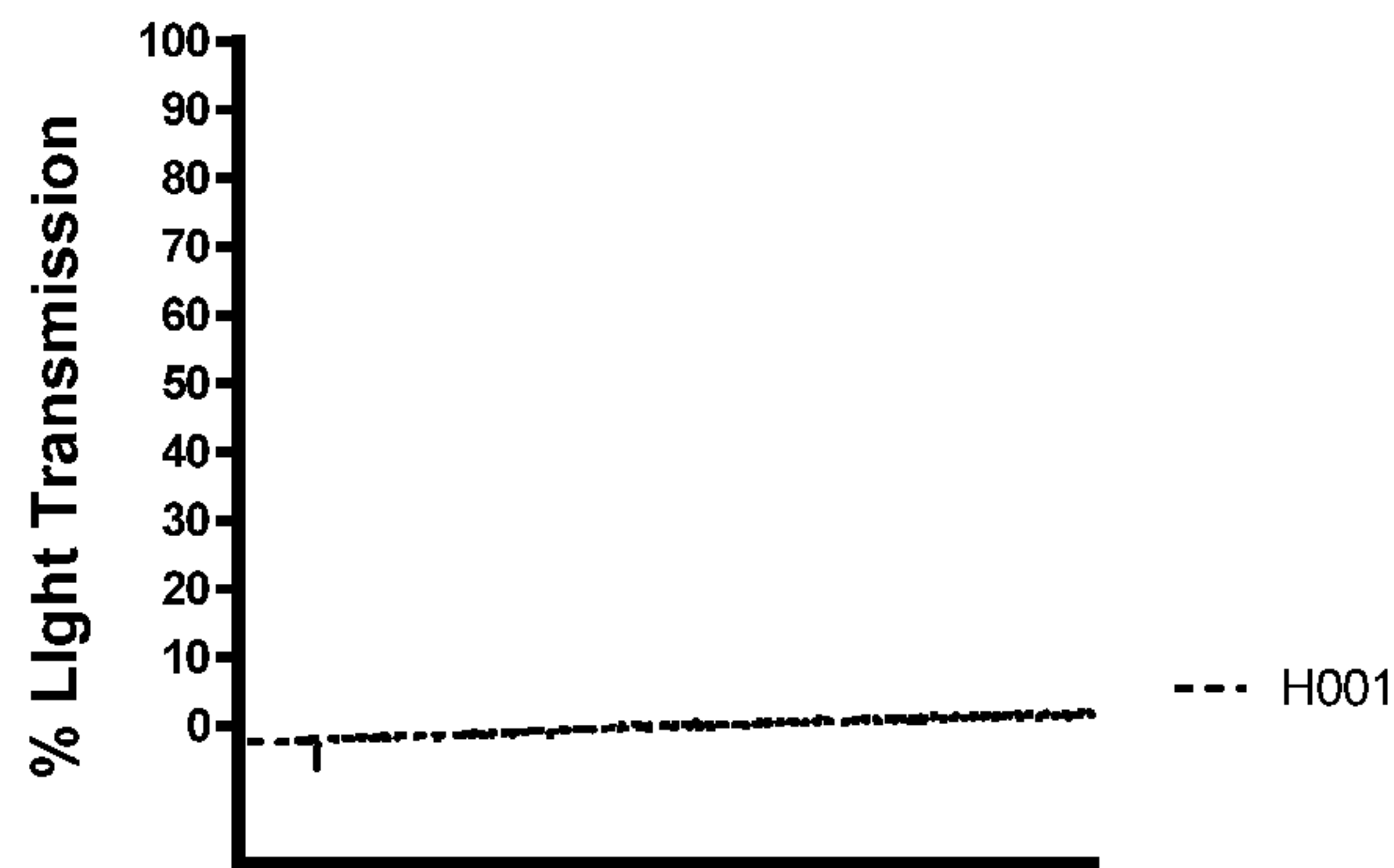
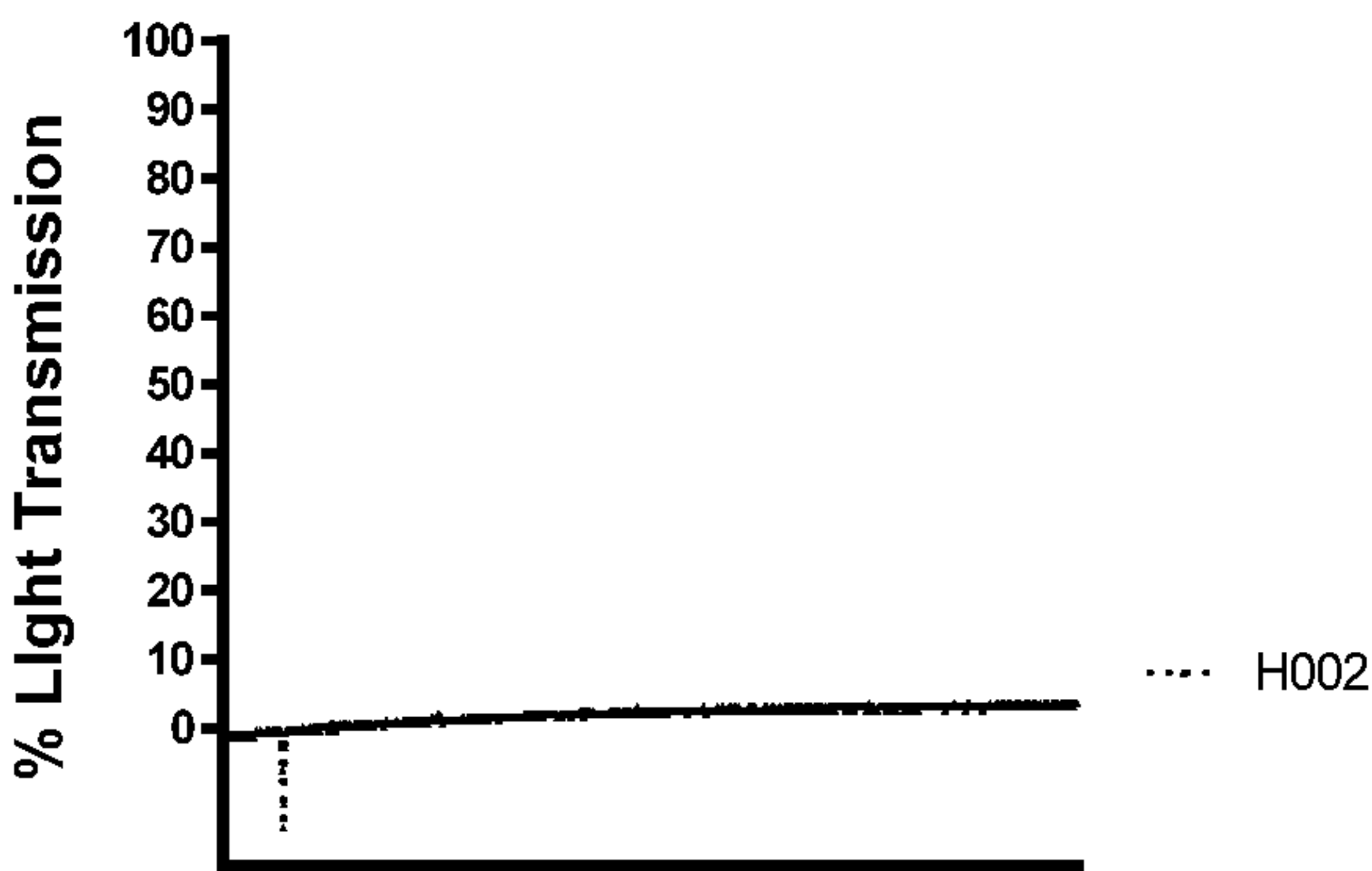


Figure 8

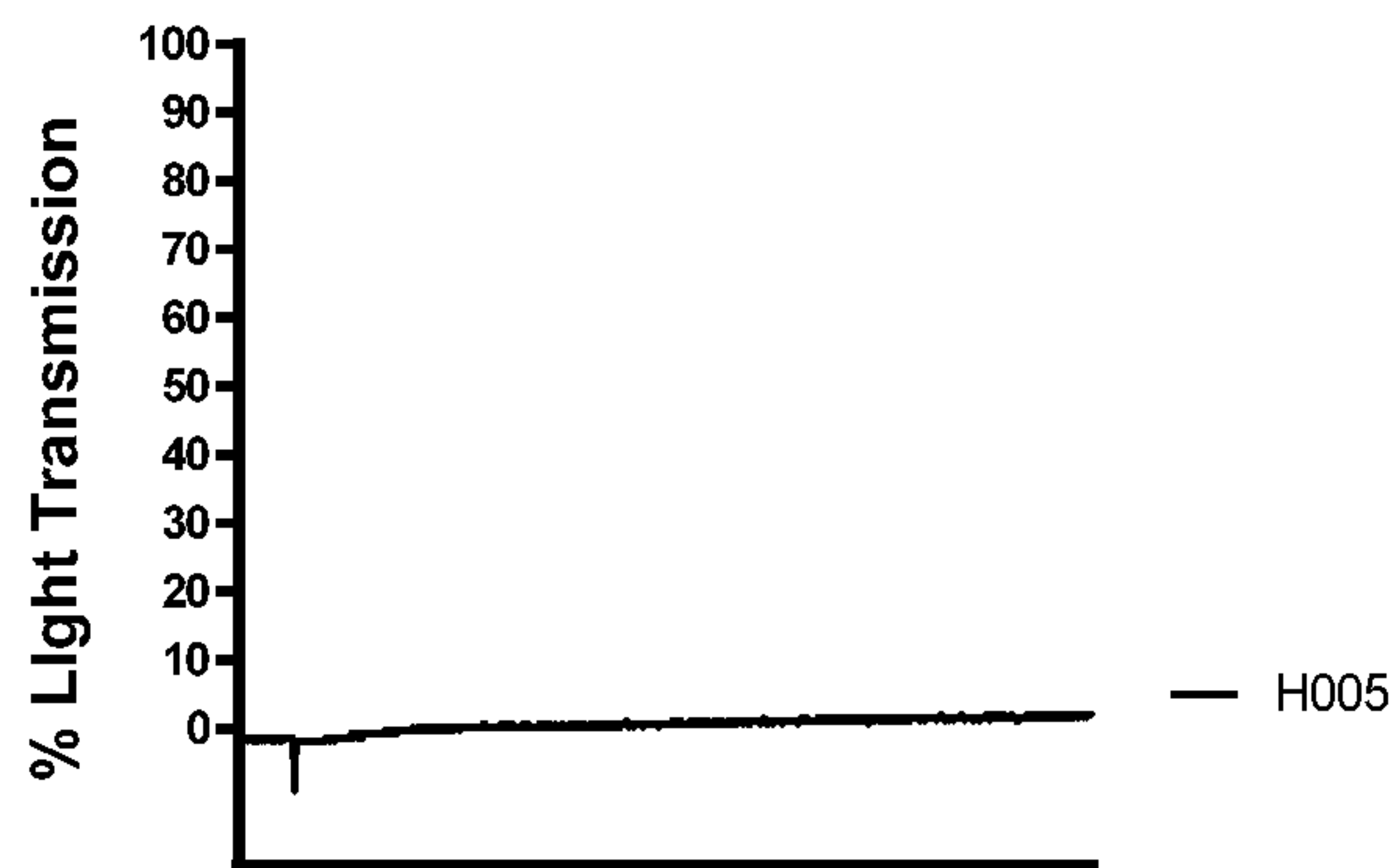
A



B



C



D

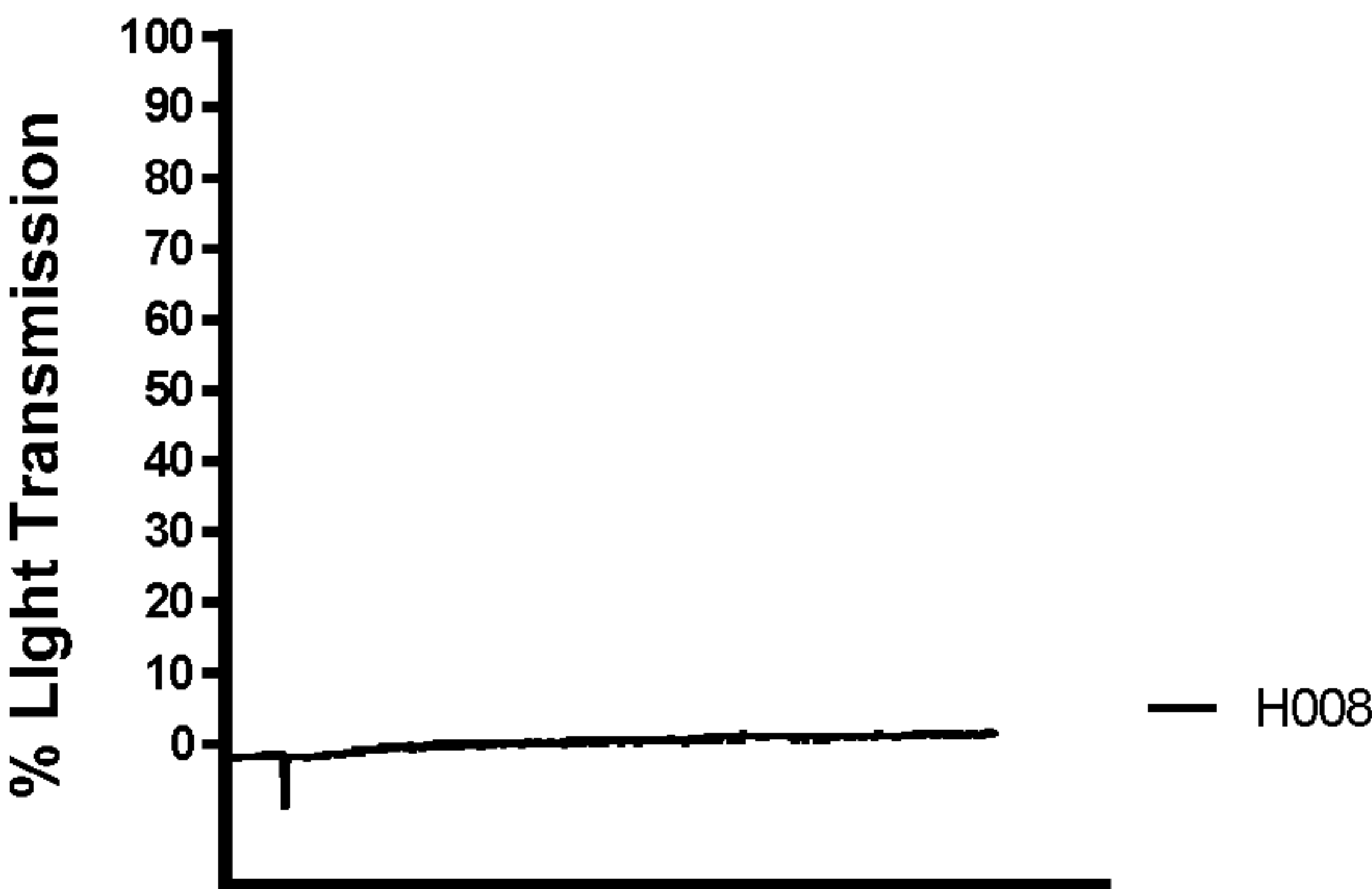
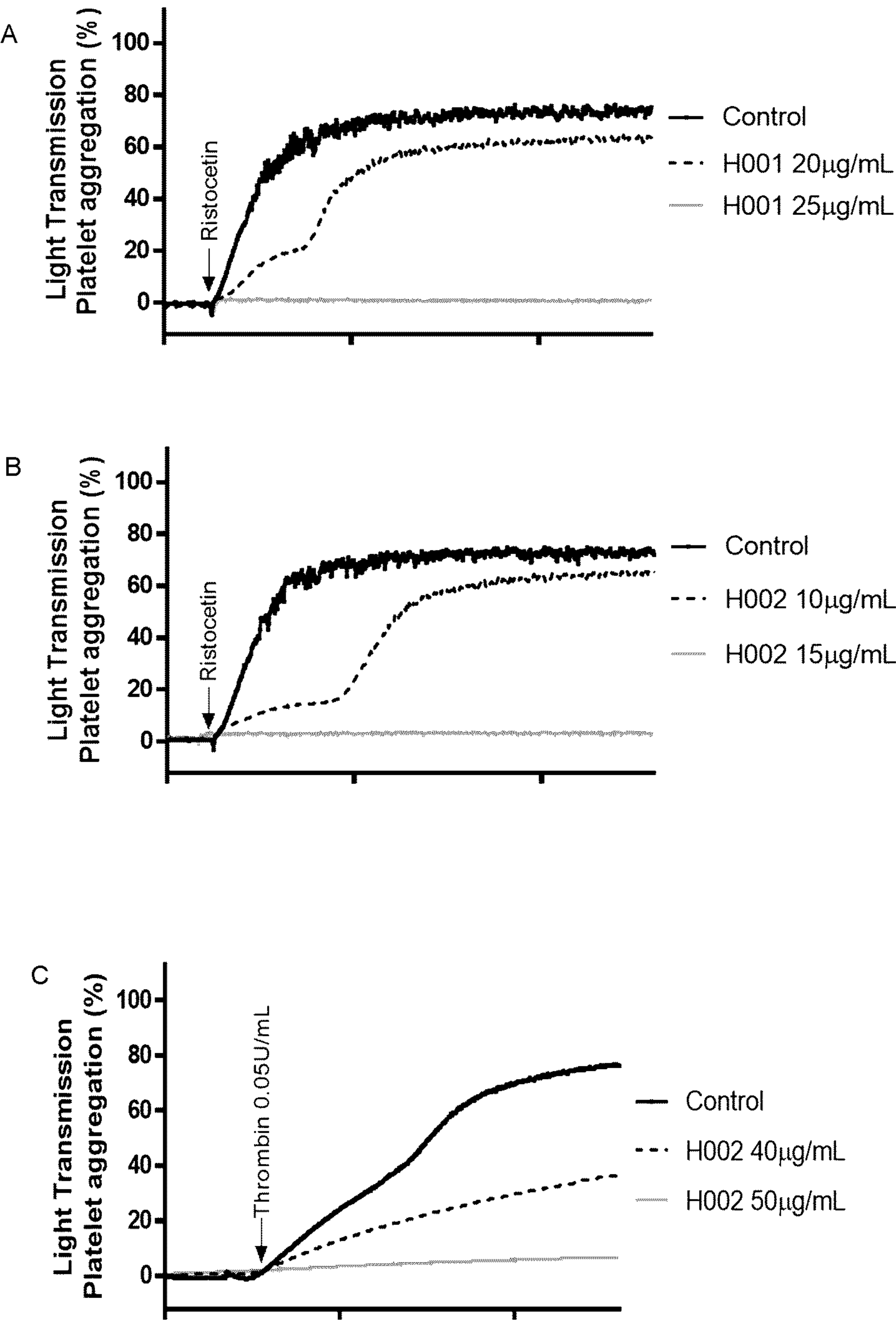
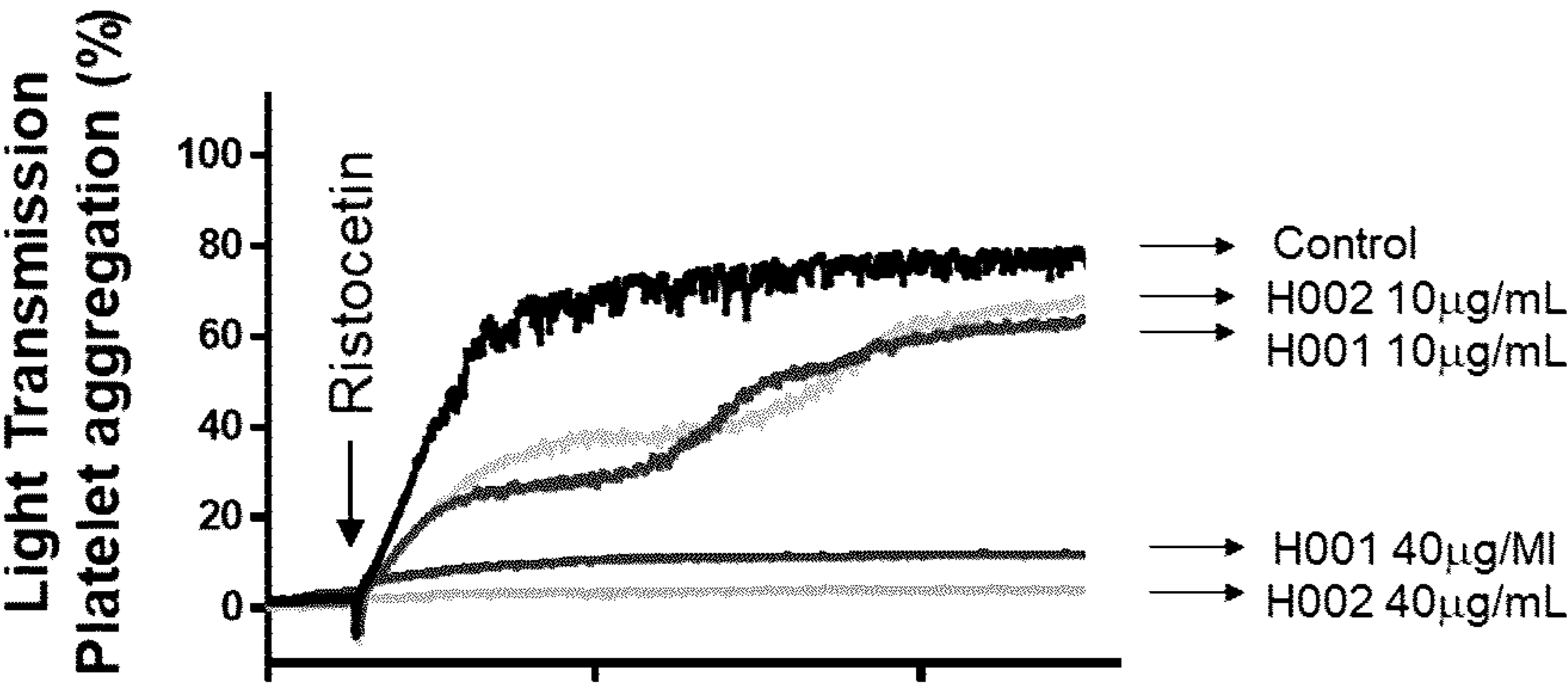


Figure 9



D



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Figure 10

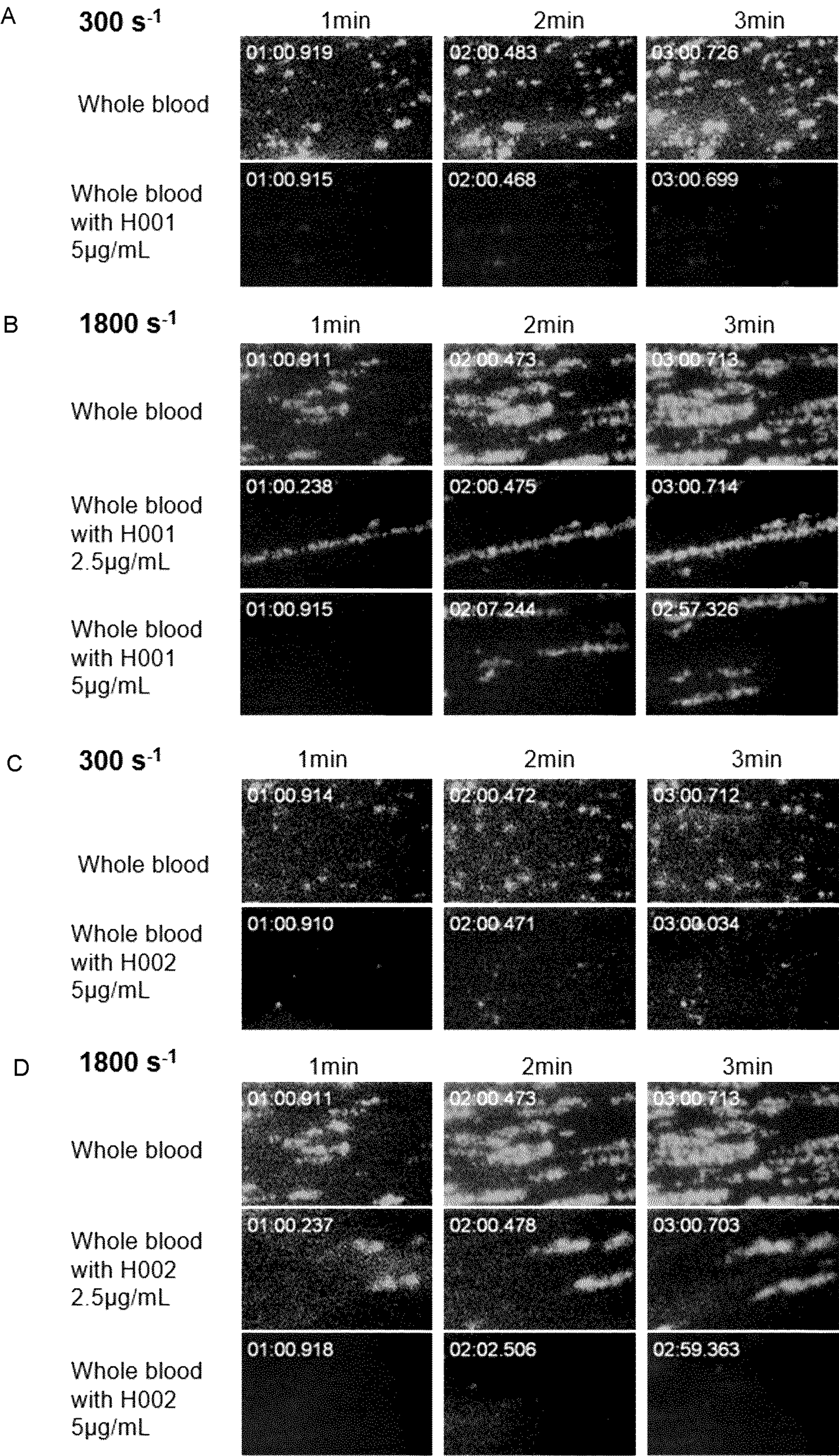
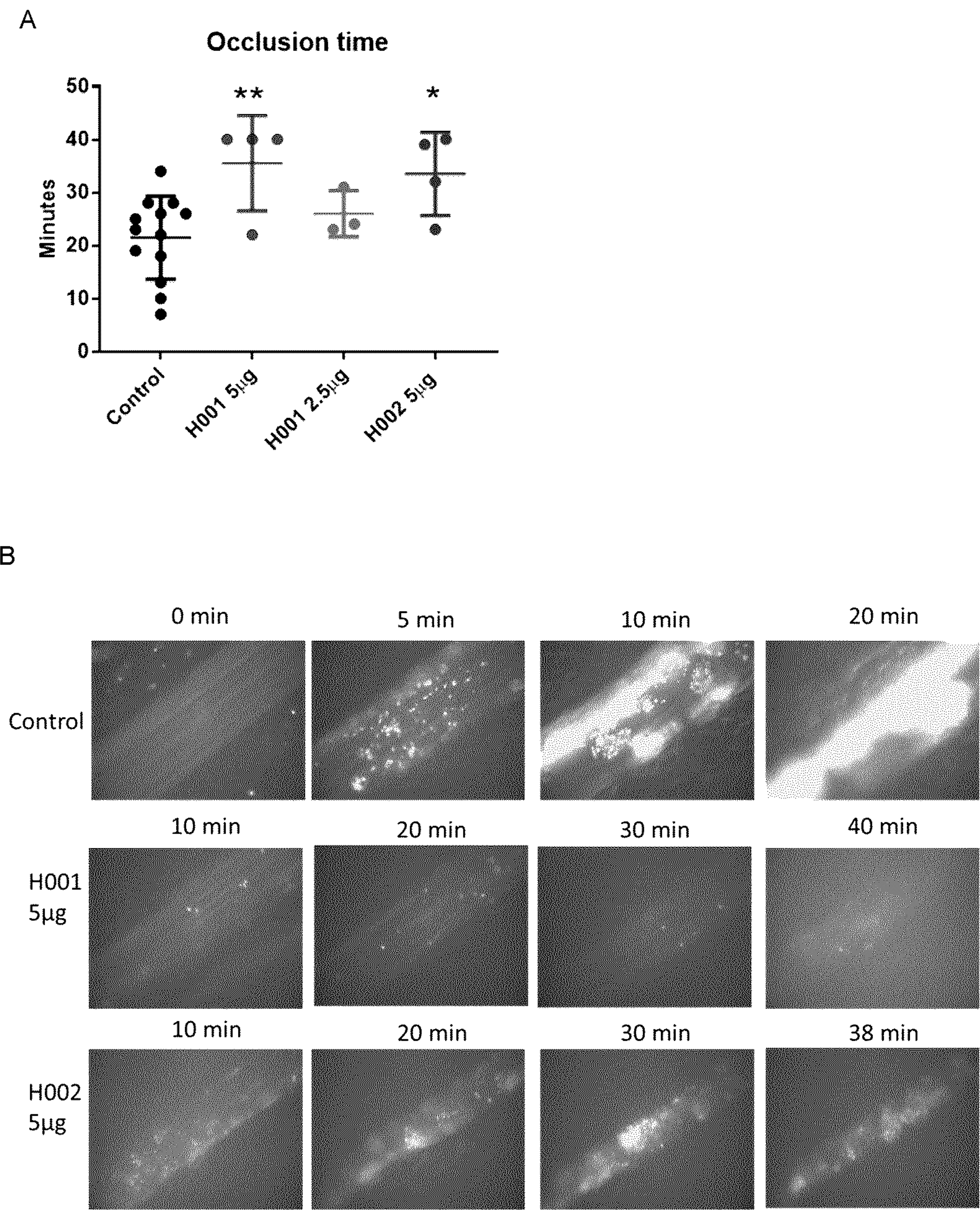


Figure 11



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Figure 12

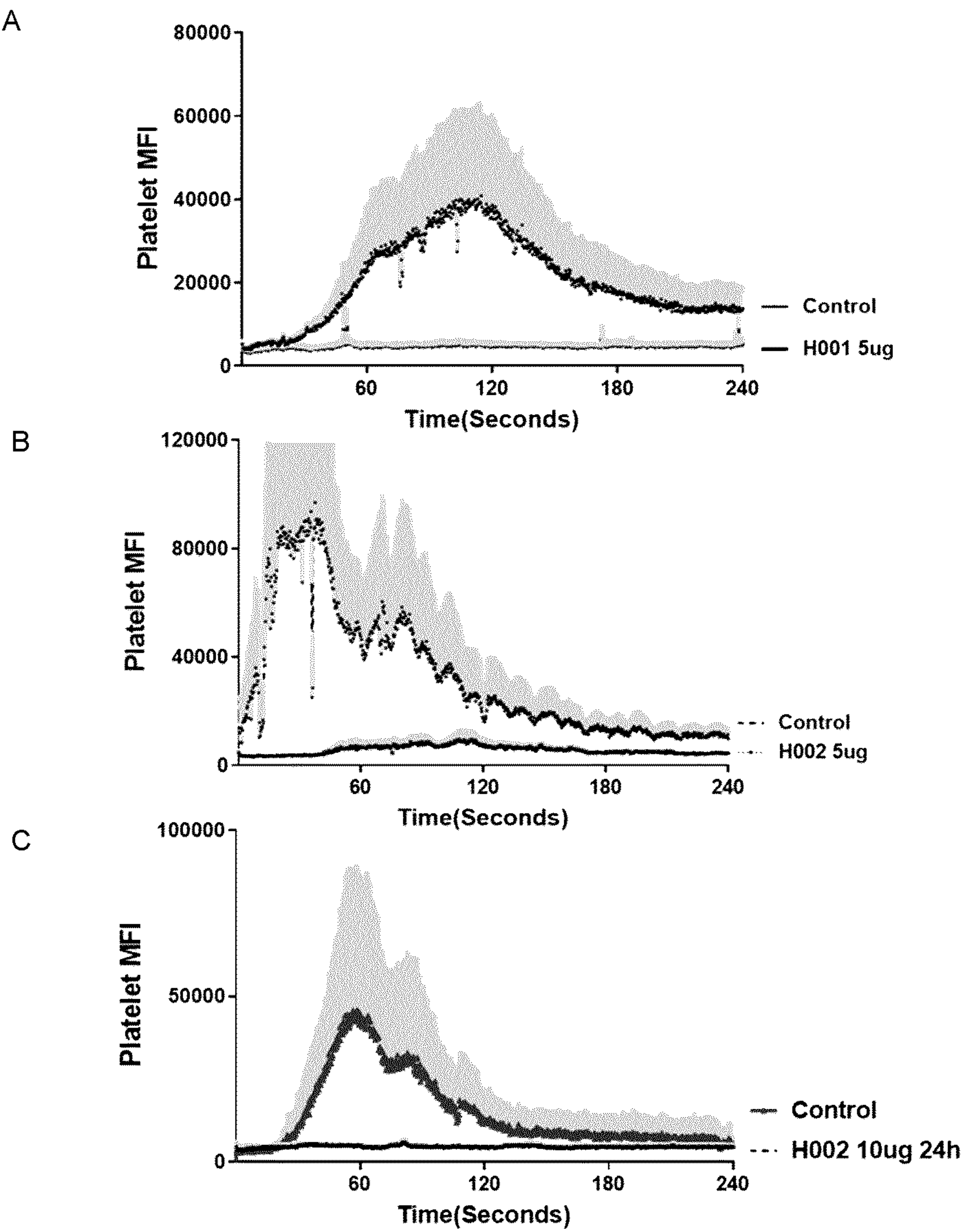
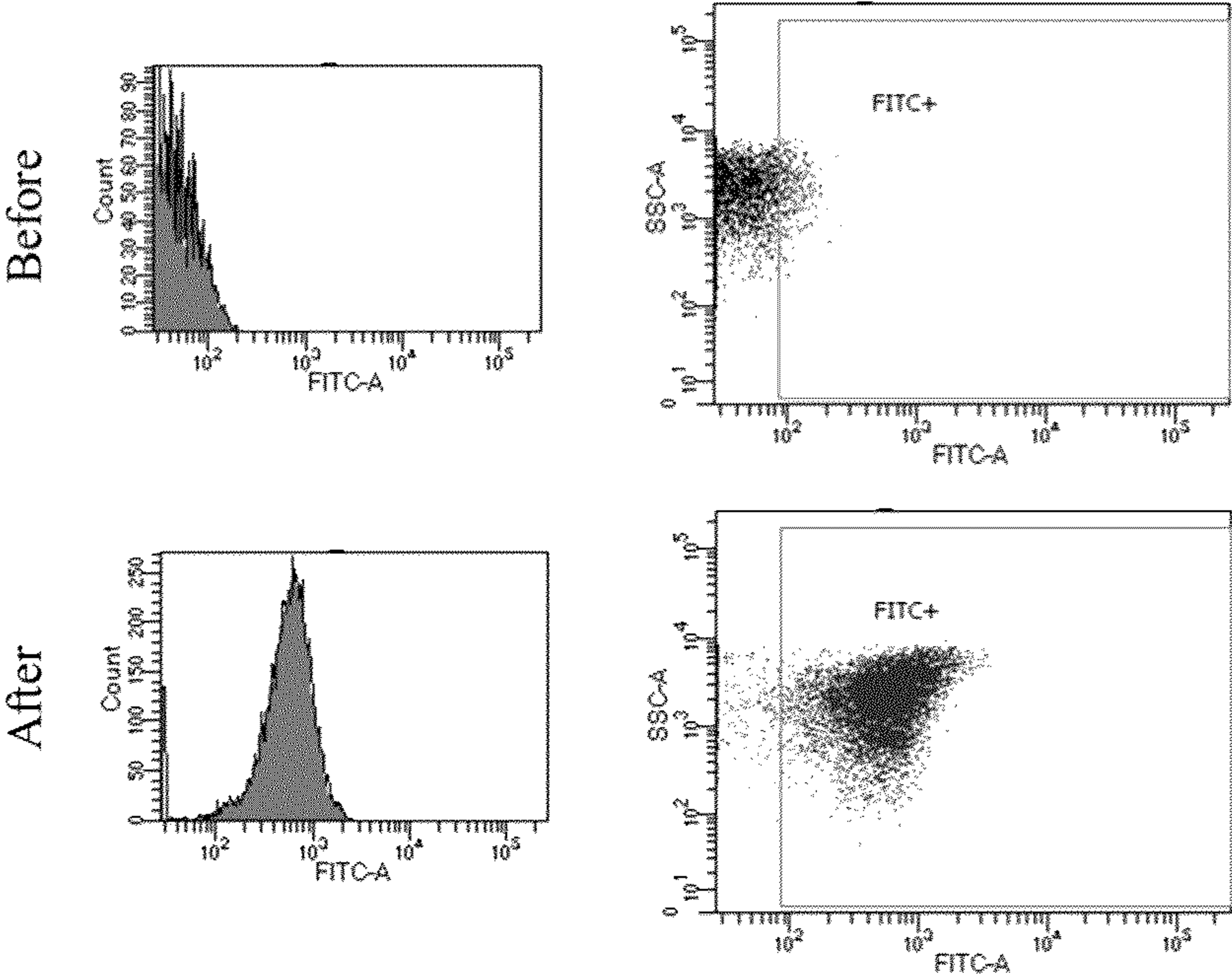
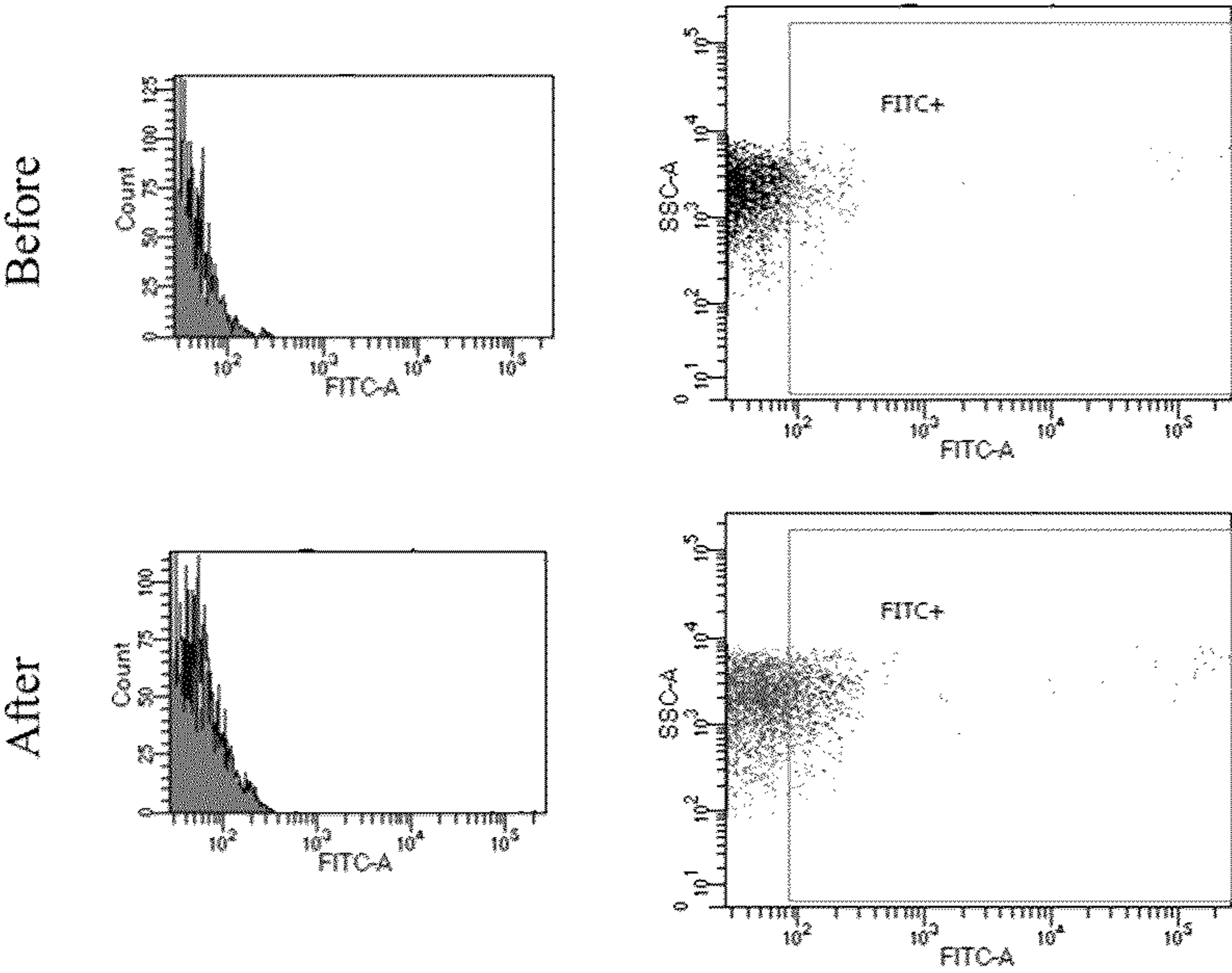


Figure 13

A H001 binds to mouse platelets in vivo



B P-selectin expression on mouse platelets



C Annexin V binding on mouse platelets

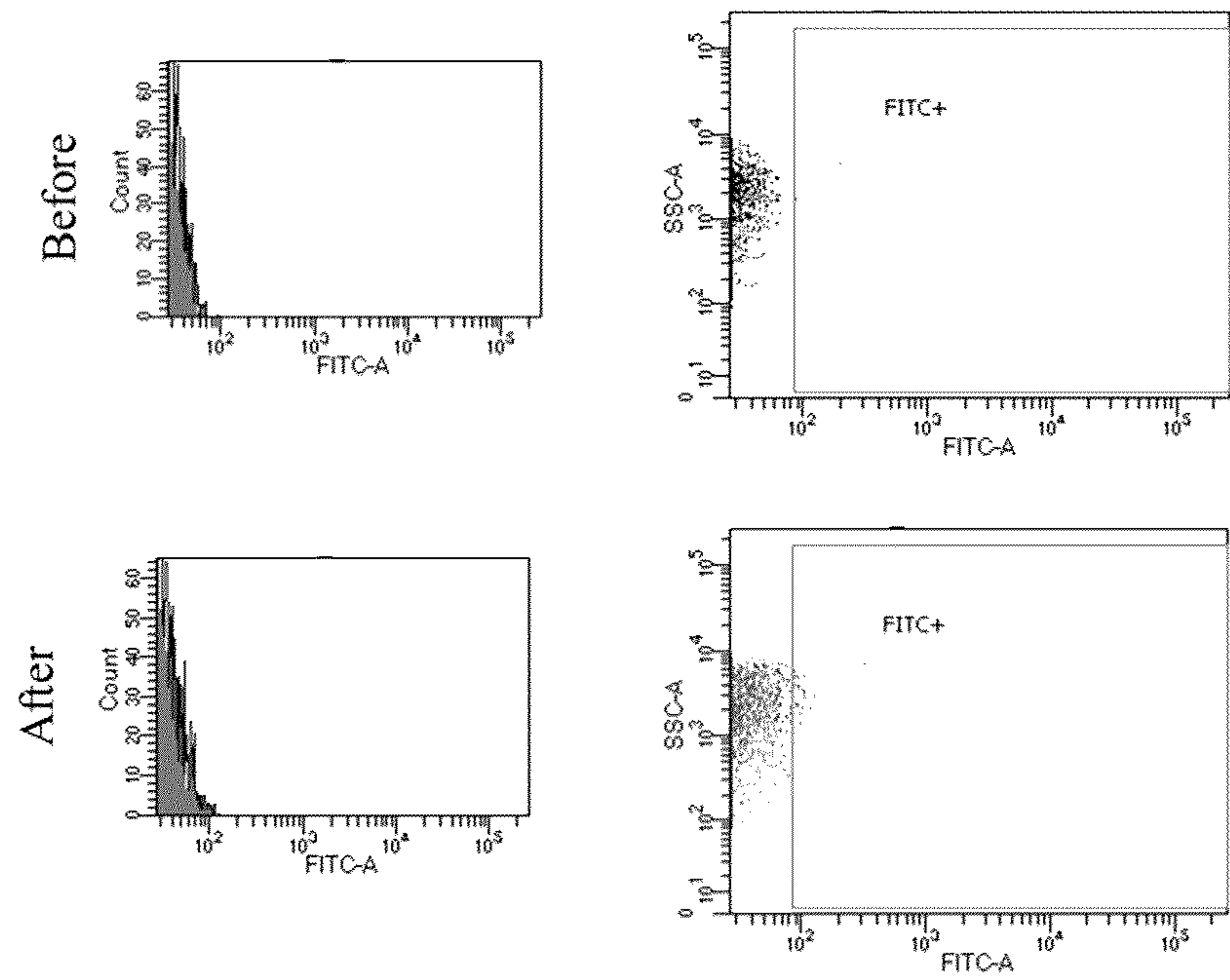


Figure 14

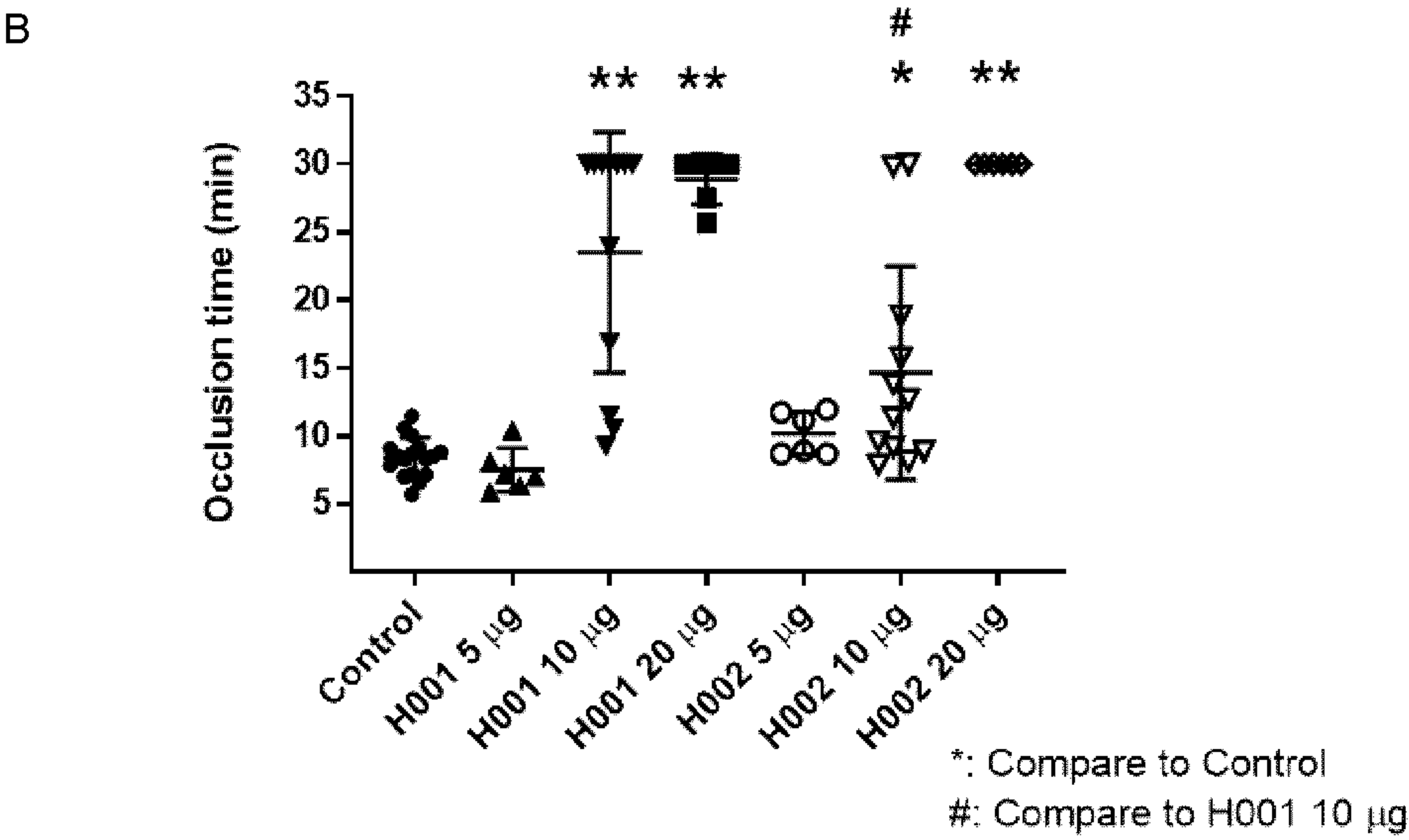
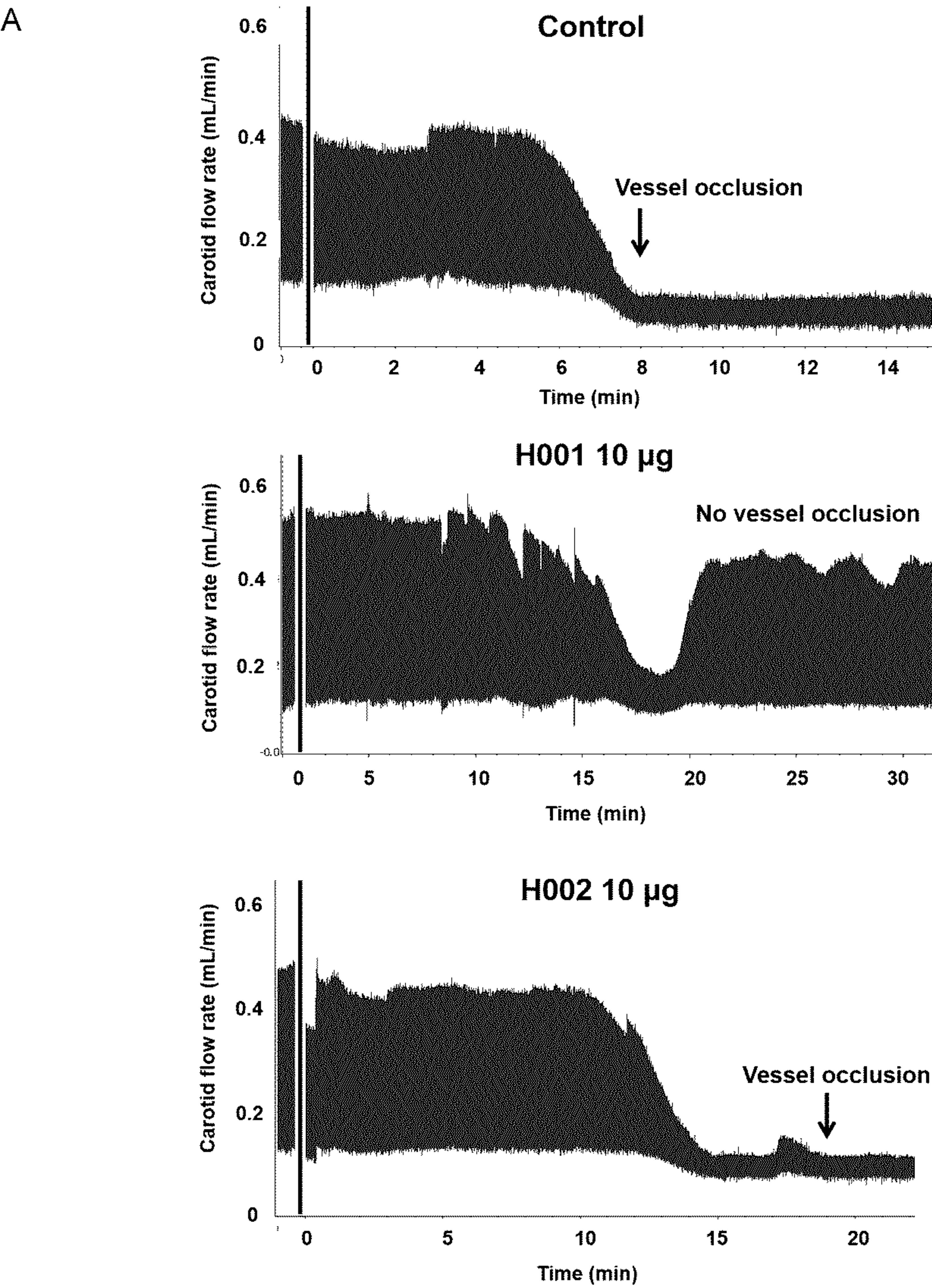


Figure 15

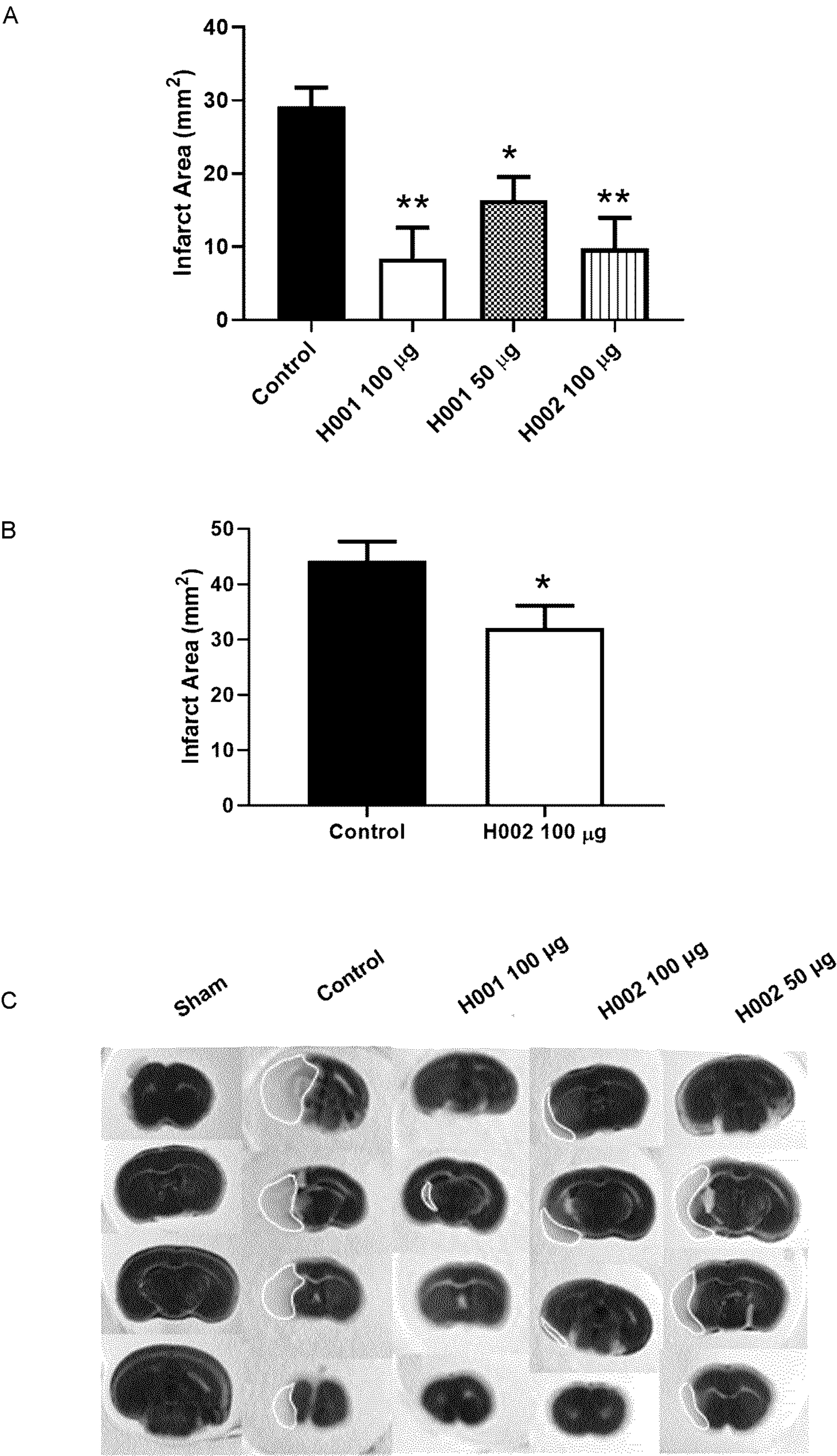


Figure 16

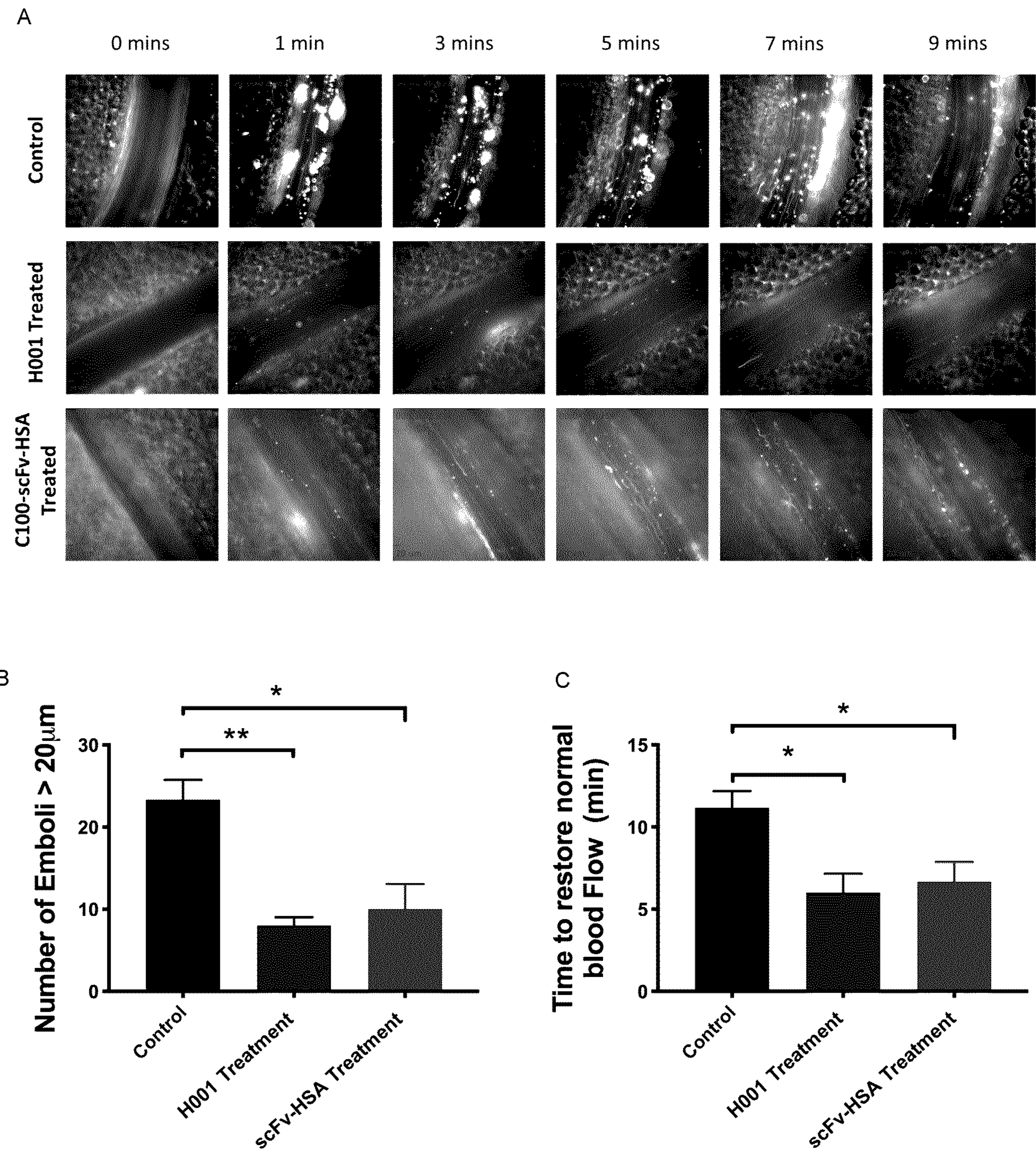


Figure 17

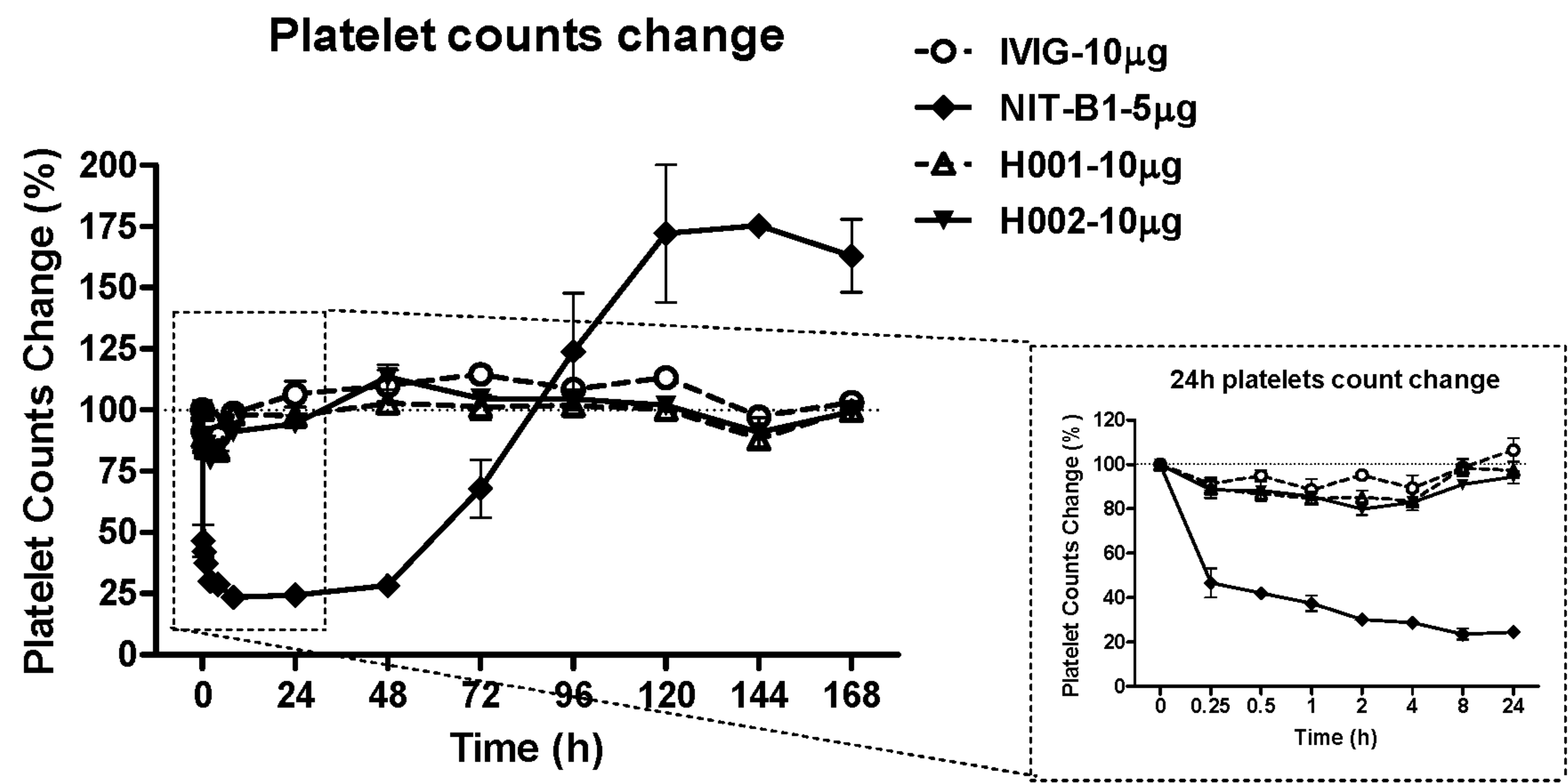


Figure 18

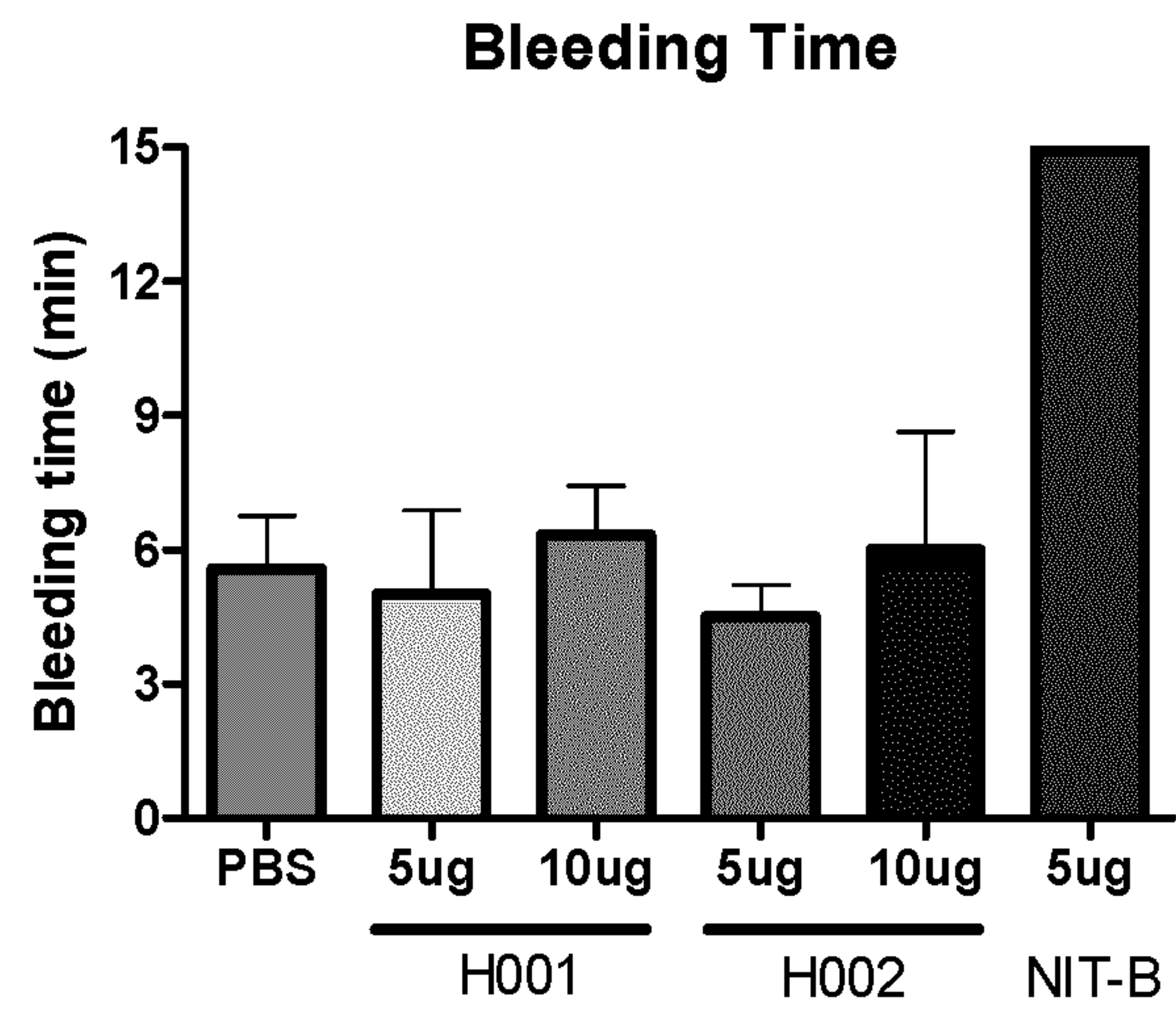
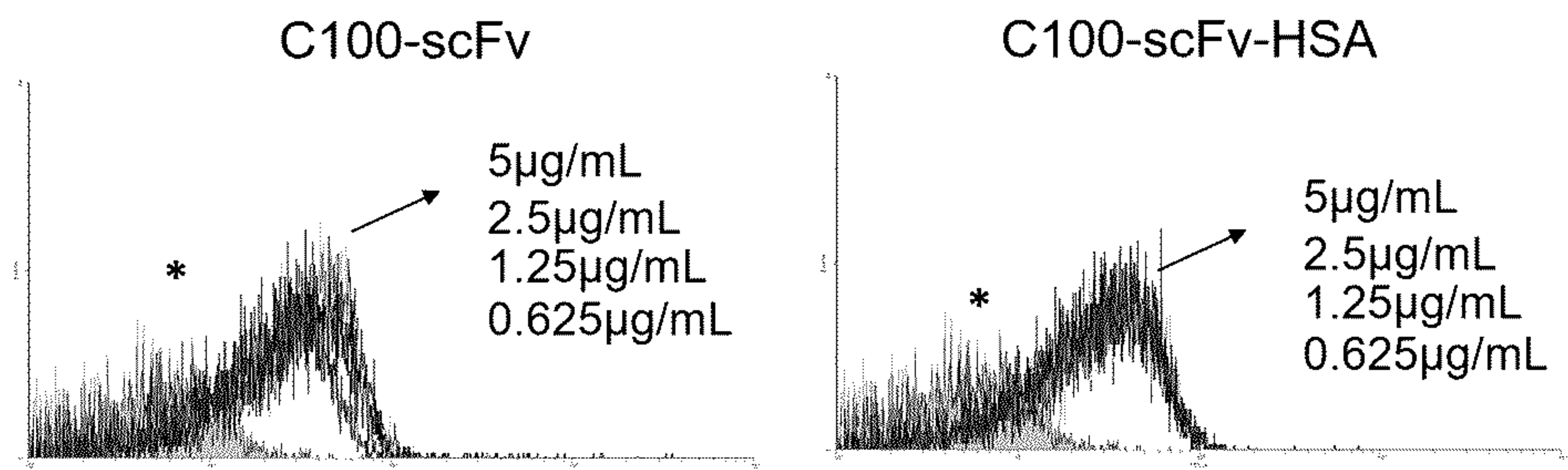
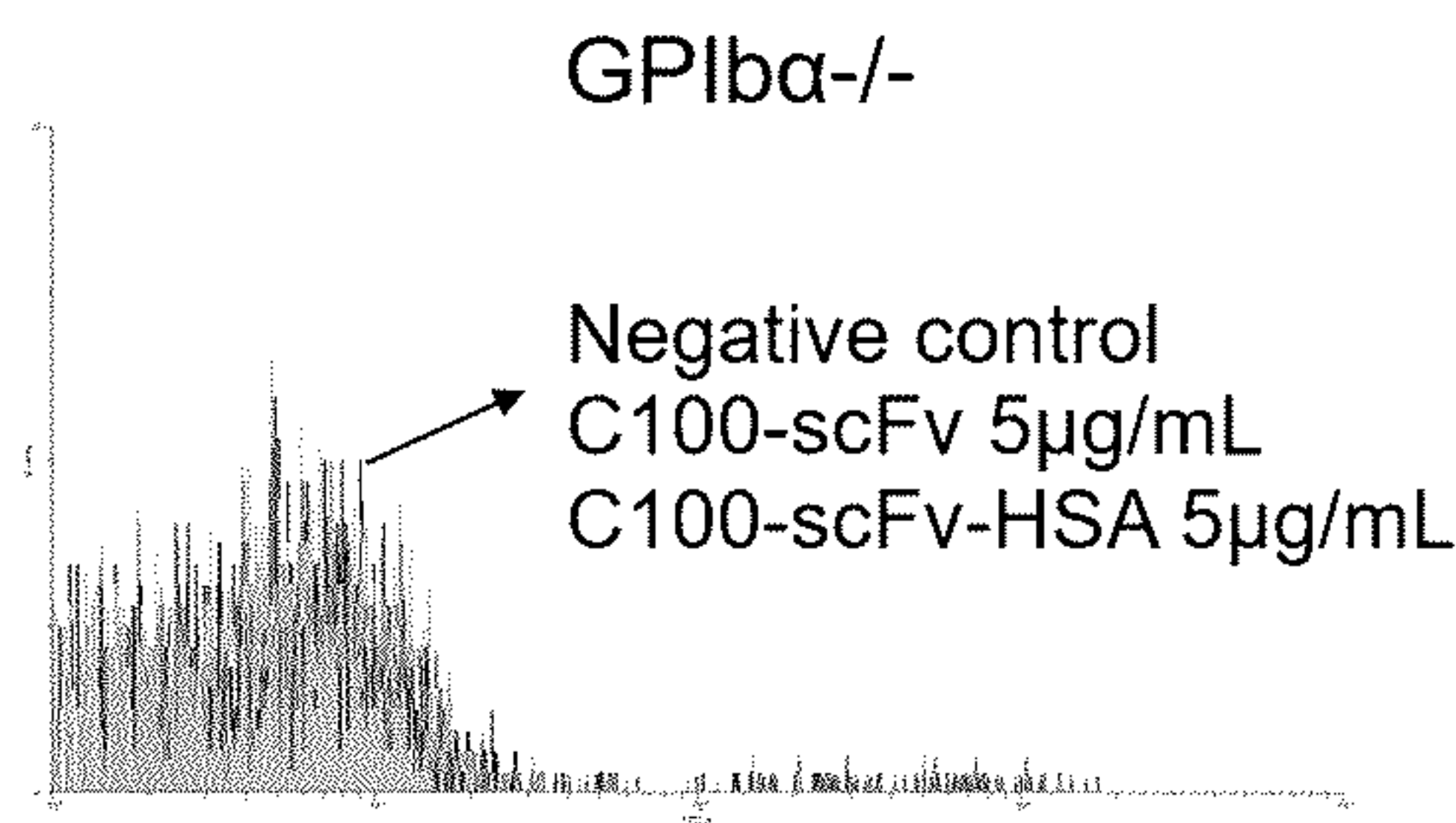


Figure 19

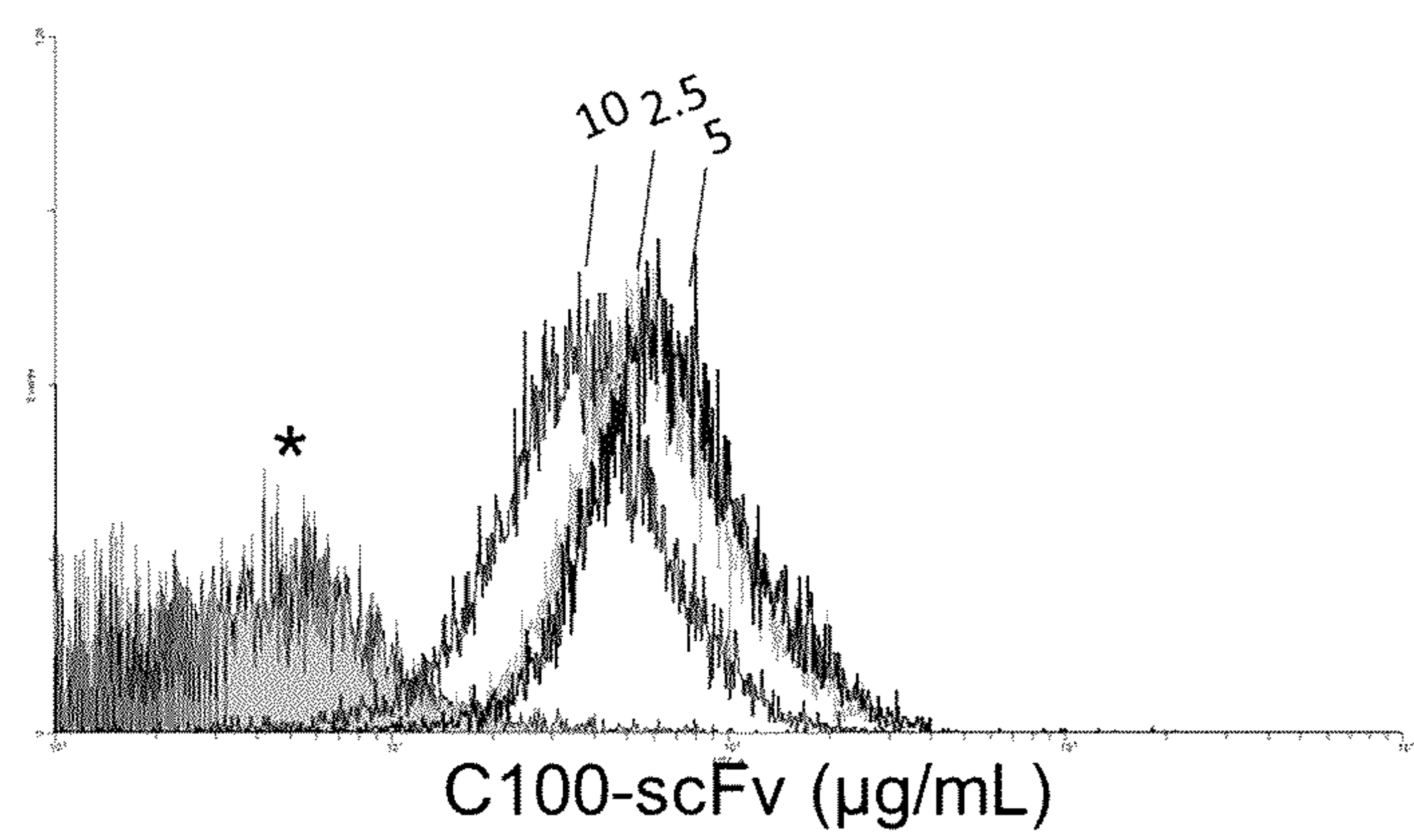
A



B



C



D

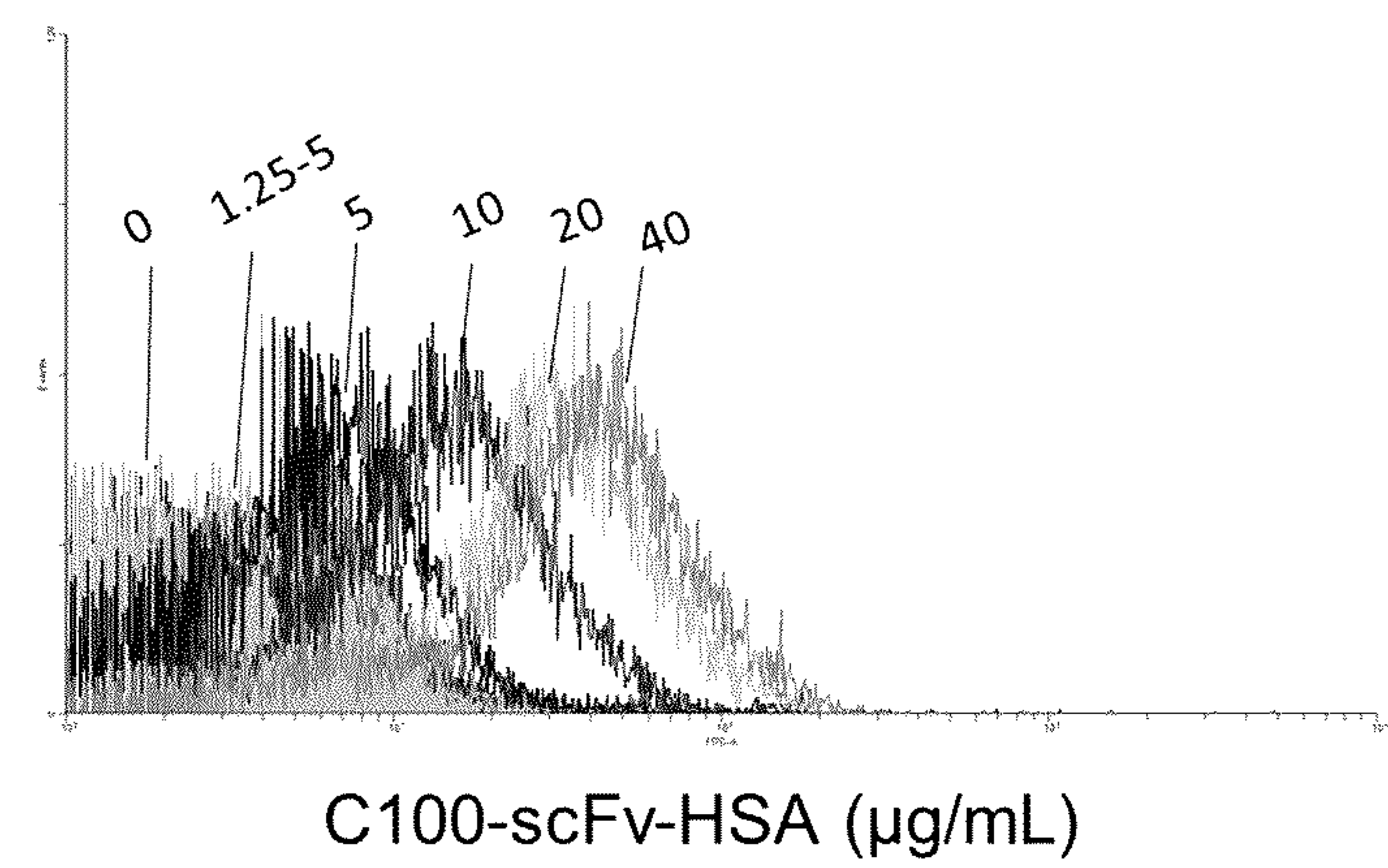


Figure 20

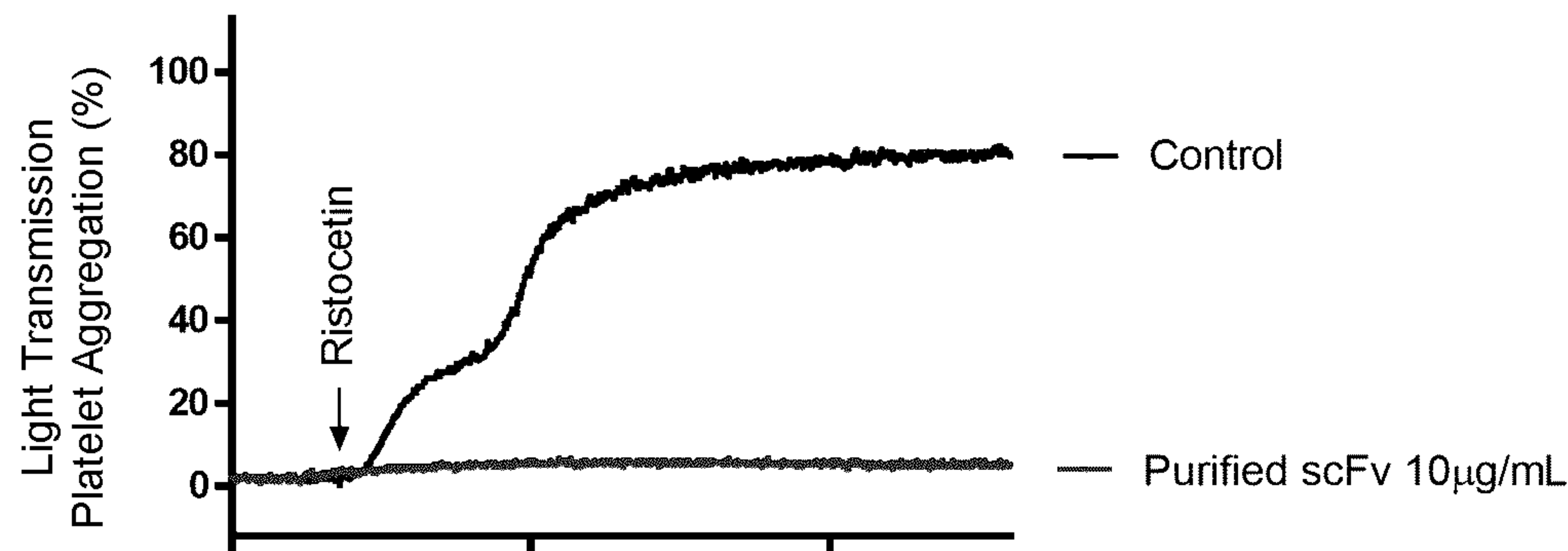


Figure 21

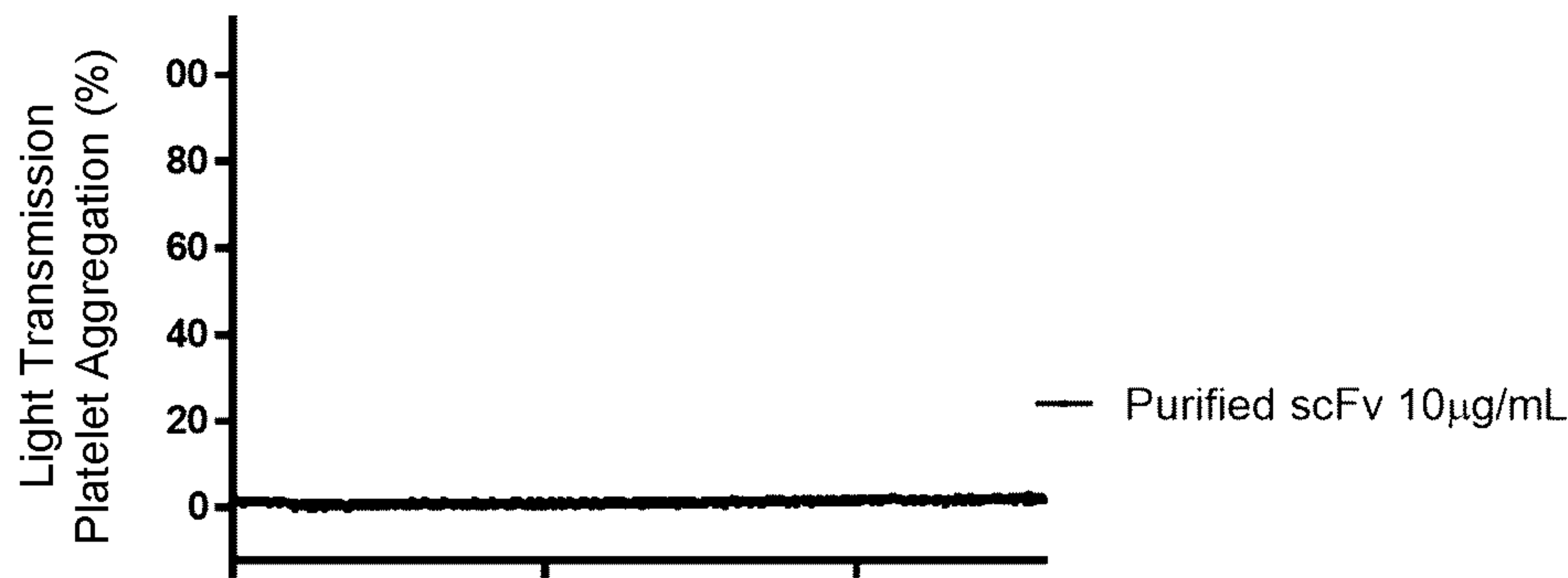
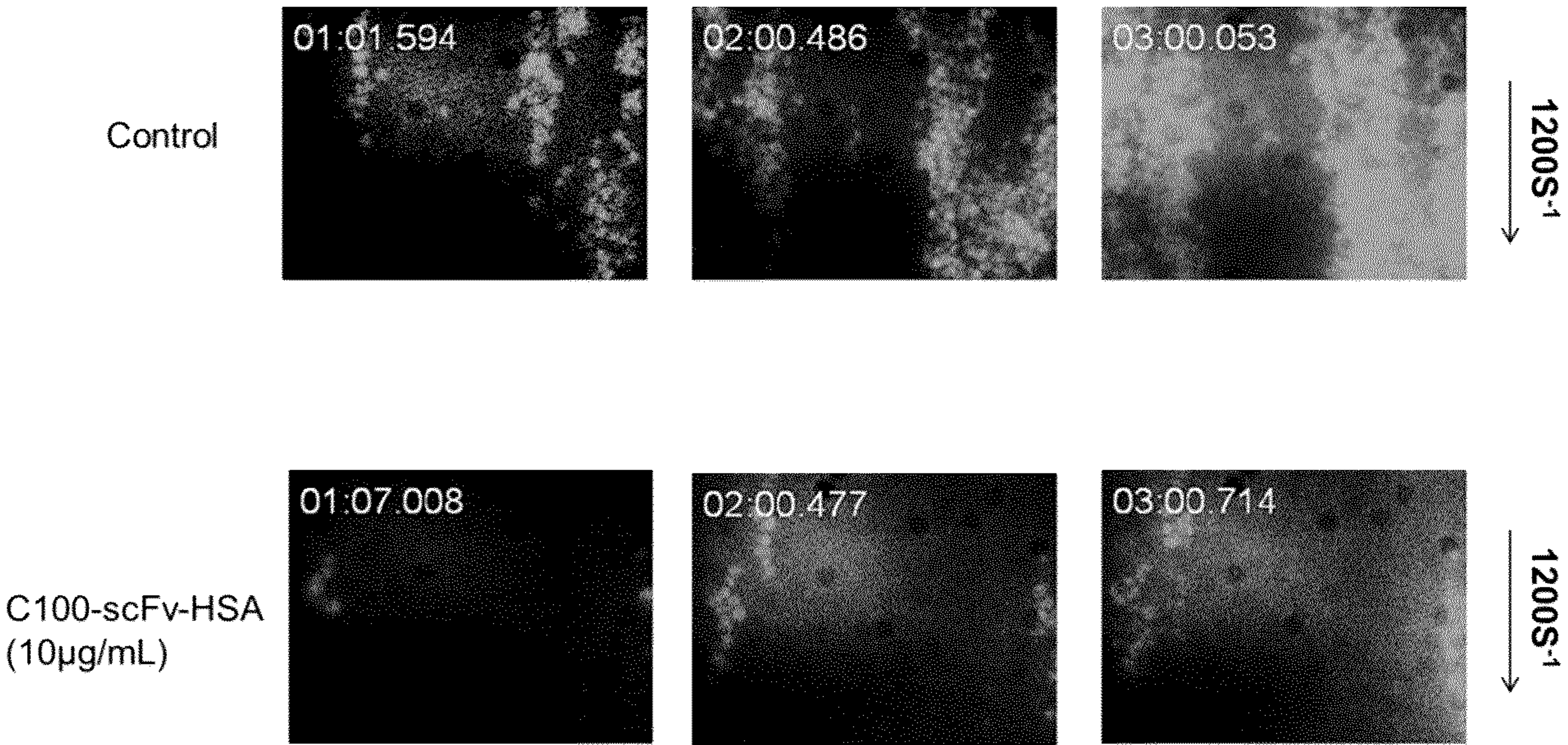


Figure 22



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2020/051699

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07K 16/46** (2006.01), **A61K 39/395** (2006.01), **A61K 39/44** (2006.01), **A61P 35/04** (2006.01),
A61P 7/02 (2006.01), **C07K 16/28** (2006.01), **C07K 19/00** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Keywords searched across the entire IPC

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Canadian Patents Database, Questel-Orbit, Scopus, PubMed, Google Scholar, GQ-Pat, PDP-Protein, ENSEMBL, Swiss-Prot, RefSeq, EMBL
Keywords: glycoprotein I(b) α (GPIb α), platelet, aggregation, antibody, humanized, Fab, NIT-B1, von Willebrand factor (VWF), tumor metastasis, SEQ ID NOS: 36-39, 43, 50, 57, 64-67, 71, 78, and 85 Inventor: Ni, Heyu

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 7,332,162 B1 (DECKMYN, H. et al.) 19 February 2008 (19-02-2008)	1-8, 10, 13, 15, 19-31
Y	*entire document*	32-33
X	US 8,323,652 B2 (NI, H. et al.) 04 December 2012 (04-12-2012)	1-31
Y	*entire document*	32-33
Y	QI, Y. et al. "Novel antibodies against GPIb α inhibit pulmonary metastasis by affecting vWF-GPIb α interaction", J. Hematol. Oncol. 17 September 2018 (17-09-2018), vol. 11, no. 1, Article number: 117, ISSN 1756-8722.	32-33
	entire document	

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
15 February 2021 (15-02-2021)

Date of mailing of the international search report
15 March 2021 (15-03-2021)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Ernest Tan (819) 639-7658

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13^{ter}.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13^{ter}.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13^{ter}.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/CA2020/051699

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US7332162B1	19 February 2008 (19-02-2008)	AT353101T AU7509300A DE60033234D1 DE60033234T2 EP1076089A1 EP1200562A2 EP1200562B1 GB9918788D0 US2009010934A1 US2009311267A1 WO0110911A2 WO0110911A3	15 February 2007 (15-02-2007) 05 March 2001 (05-03-2001) 22 March 2007 (22-03-2007) 08 November 2007 (08-11-2007) 14 February 2001 (14-02-2001) 02 May 2002 (02-05-2002) 31 January 2007 (31-01-2007) 13 October 1999 (13-10-1999) 08 January 2009 (08-01-2009) 17 December 2009 (17-12-2009) 15 February 2001 (15-02-2001) 21 June 2001 (21-06-2001)
US8323652B2	04 December 2012 (04-12-2012)	US2010150837A1 CA2689726A1 CA2689726C DK2186829T3 EP2186829A1 EP2186829B1	17 June 2010 (17-06-2010) 14 May 2010 (14-05-2010) 16 September 2014 (16-09-2014) 07 April 2015 (07-04-2015) 19 May 2010 (19-05-2010) 31 December 2014 (31-12-2014)