Supplementary Material

Platelet bio-nanobubbles as microvascular recanalization nanoformulation for the acute ischemic stroke lesion theranostics

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The supplementary material includes:

Figures S1-12

Tables S1-2

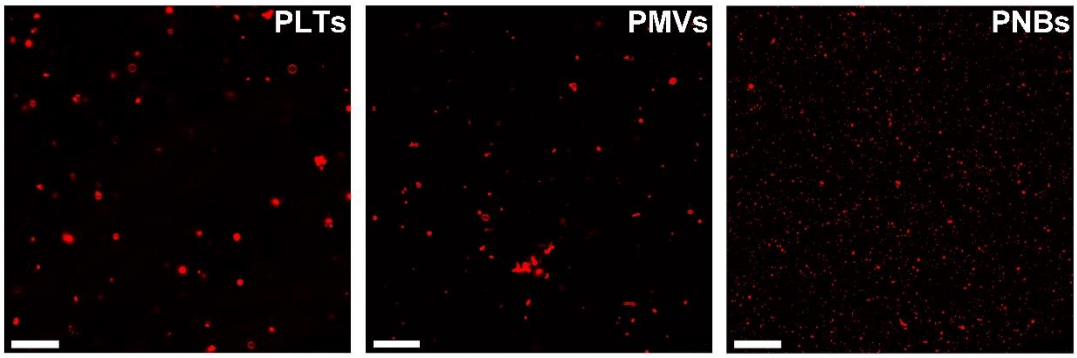

Figure S1. Confocal fluorescent images of PLTs, PMVs, PNBs. Scale bar, 25 μm.

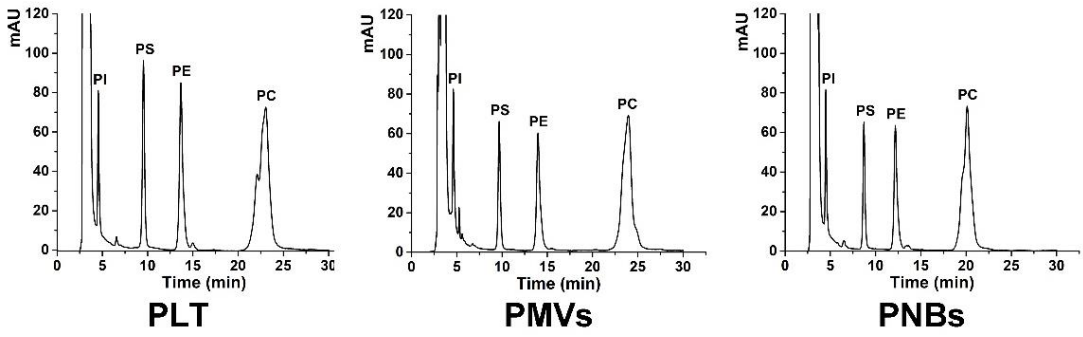

Figure S2. The HPLC chromatograms of four main phospholipids of phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) in PLT, PMVs and PNBs.

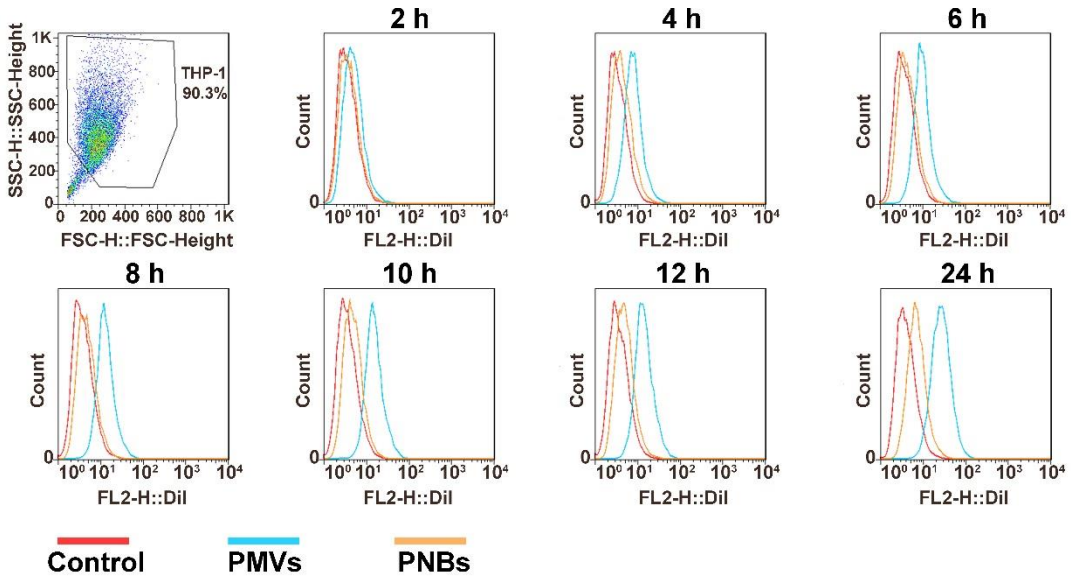

Figure S3. Flow cytometry measurement of cell uptake efficiency. Normalized fluorescence intensity of DiI-labeled PMVs (blue color) and PNBs (orange color) uptake by human THP-1 macrophage-like cells after different incubation time (2, 4, 6, 8, 10, 12, and 24 h) based on flow cytometric analysis (n= 3).

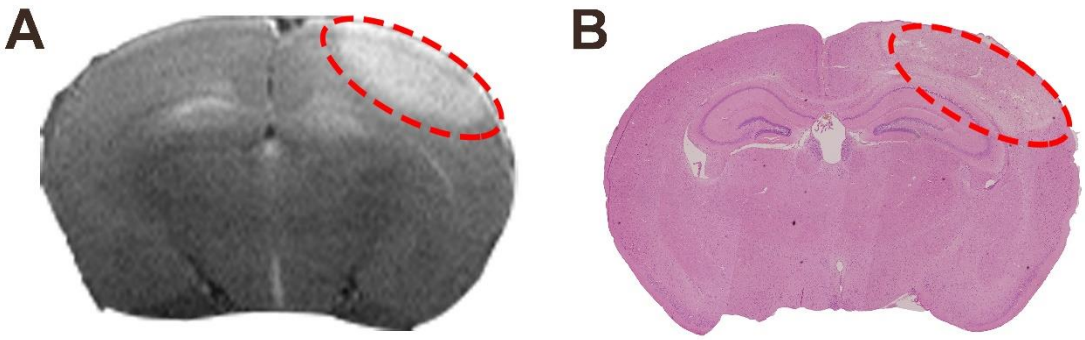

Figure S4. (A) Magnetic resonance imaging of the brain for the stroke mouse. **(B)** Hematoxylin/ eosin (HE) staining of the whole brain for the stroke mouse.

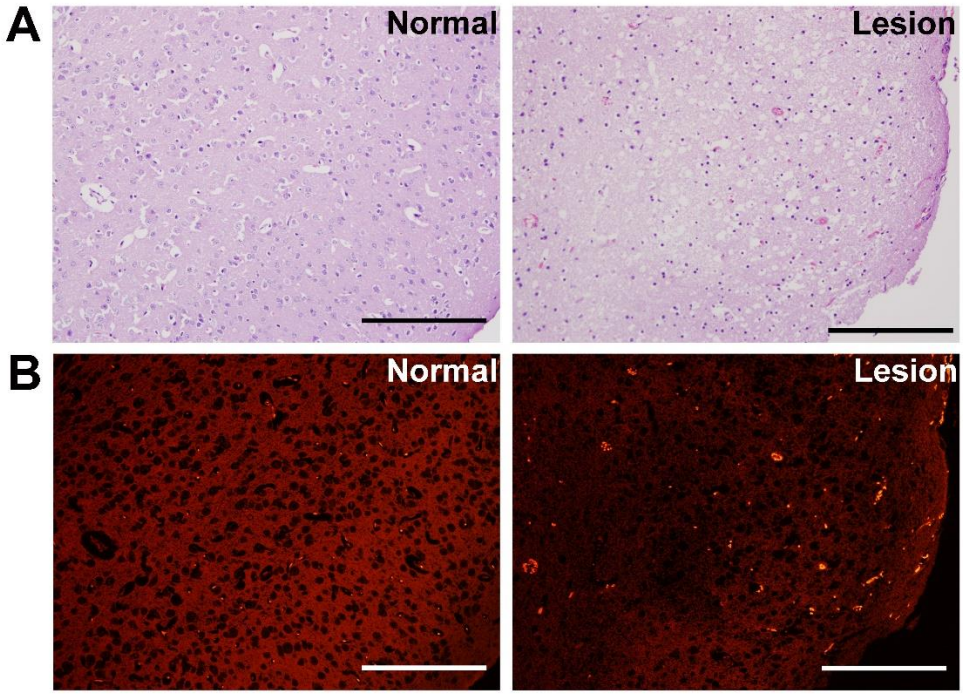

Figure S5. Histological analysis of the brain stroke 8 h post-injection of DiI-labeled PNBs. **(A)** Optical bright field images of normal brain tissue and stroke lesion brain tissue. **(B)** Fluorescence images of normal brain tissue and stroke lesion brain tissue. All scale bars, 200 μm.

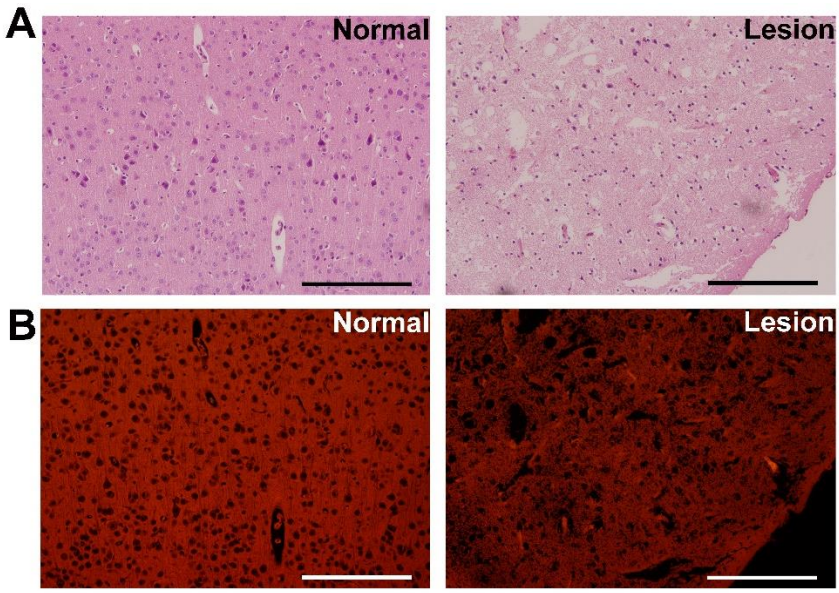

Figure S6. Histological analysis of the brain stroke 8 h post-injection of DiI-labeled PMVs. **(A)** Optical bright field images of normal brain tissue and stroke lesion brain tissue. **(B)** Fluorescence images of normal brain tissue and stroke lesion brain tissue. All scale bars, 200 μm.

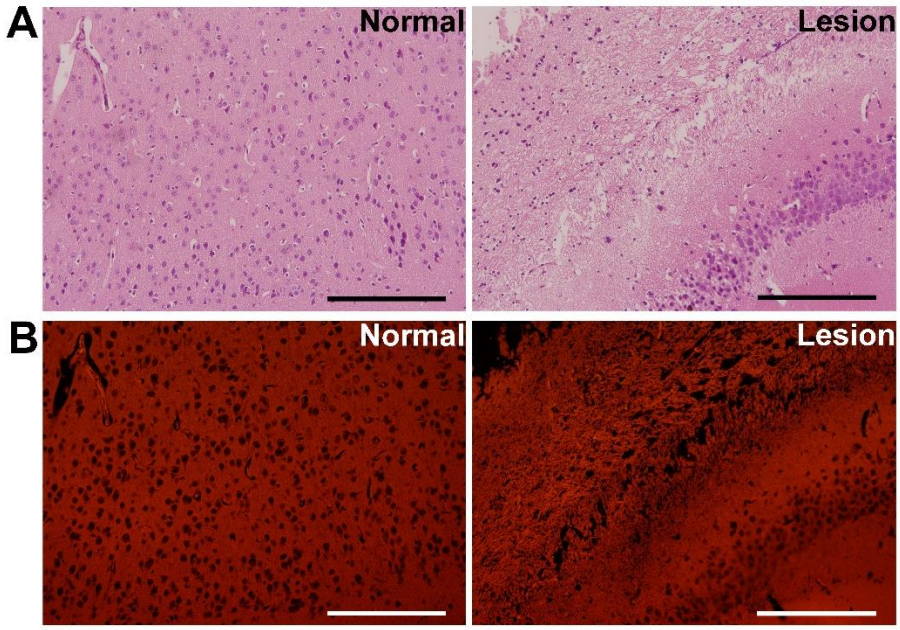

Figure S7. Histological analysis of the brain stroke 8 h post-injection of PBS. **(A)** Optical bright field images of normal brain tissue and stroke lesion brain tissue. **(B)** Fluorescence images of normal brain tissue and stroke lesion brain tissue. All scale bars, 200 μm.

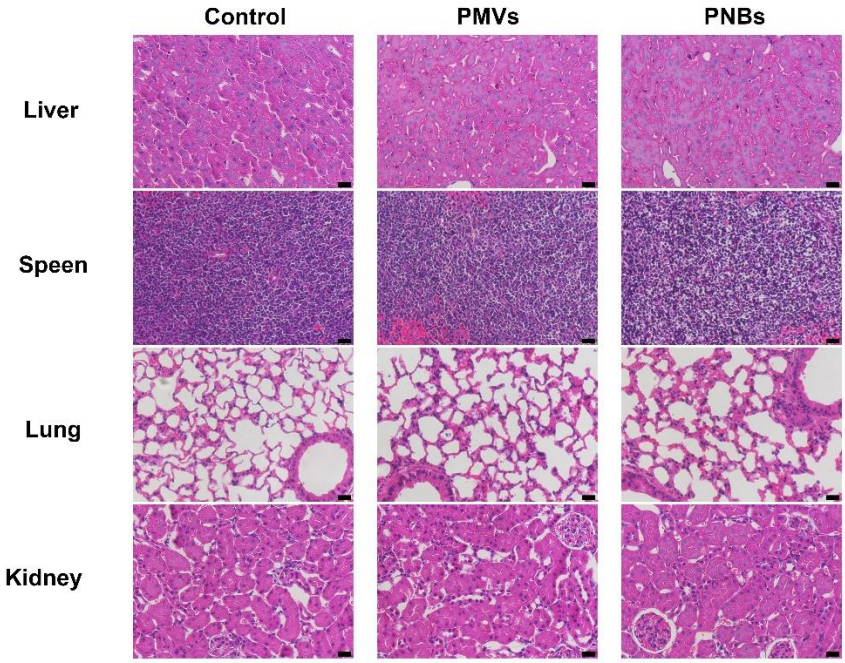

Figure S8. Histological analysis of excised major organs (liver, spleen, lung, and kidney) at 8 h after PBS, PMVs, PNBs injection.

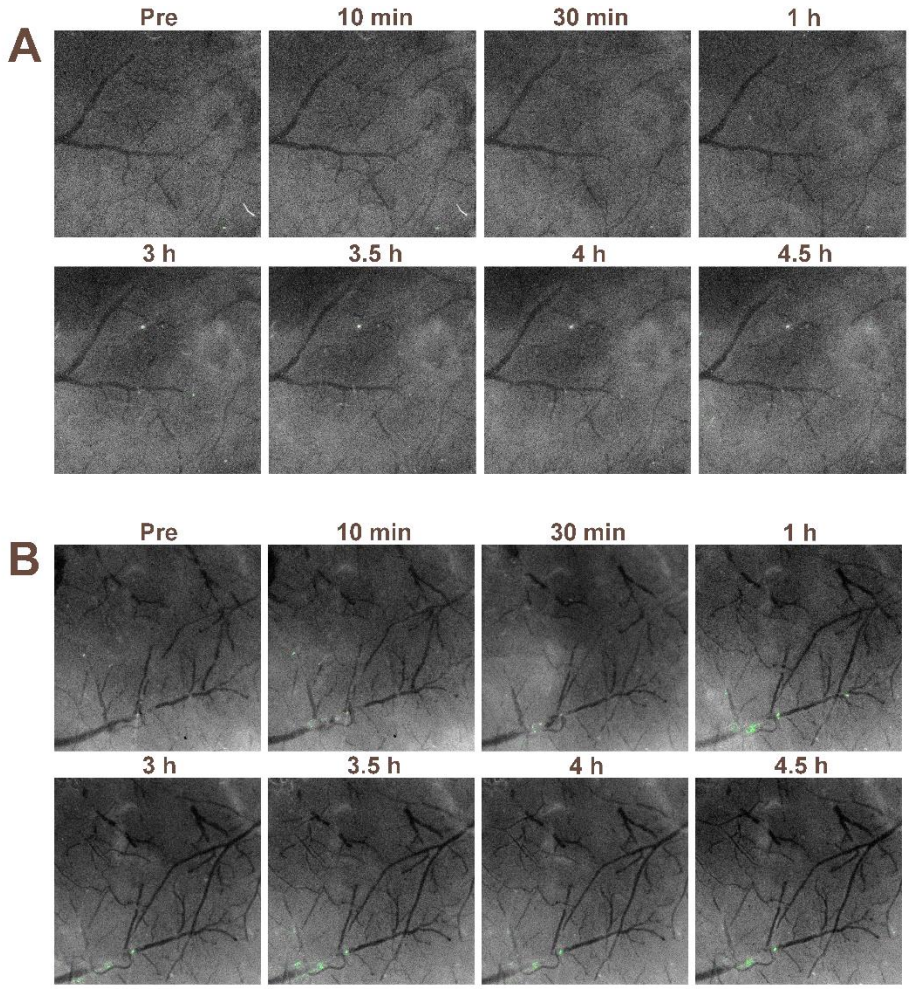

Figure S9. Histological analysis of the brain stroke after injected with PBS and DiI-labeled PMVs. Fluorescence and bright field merged stroke vascular vessle images obtained following PBS-DiO **(A)** and DiO-labled PMVs **(B)** injection.

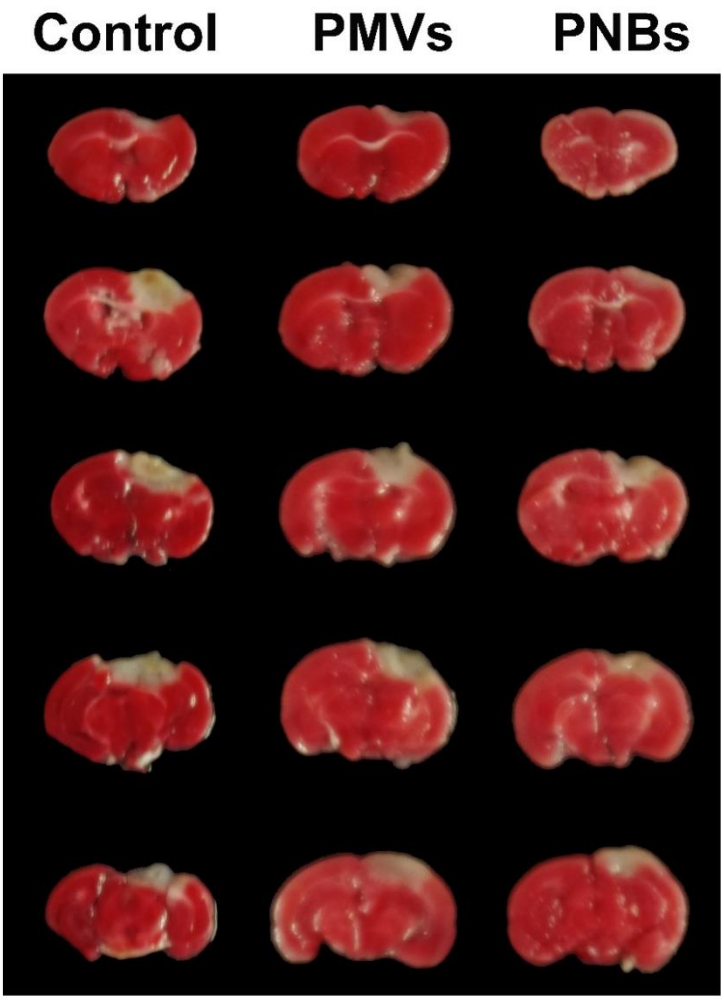

Figure S10. The images of brain coronal sections captured by the scanner. Distal photosensitive dye induced stroke occlusion caused infarct (white in color) in the ipsilateral cortex. Mice in PNBs pre-treated group showed less brain lesion infarct.

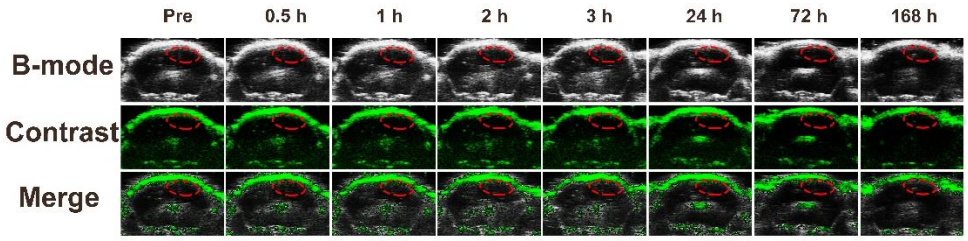

Figure S11. *In vivo* ultrasound images of mice brain before (0 h) and after PBS injection at different time points (0.5, 1, 2, 3, 24, 72, 168 h).

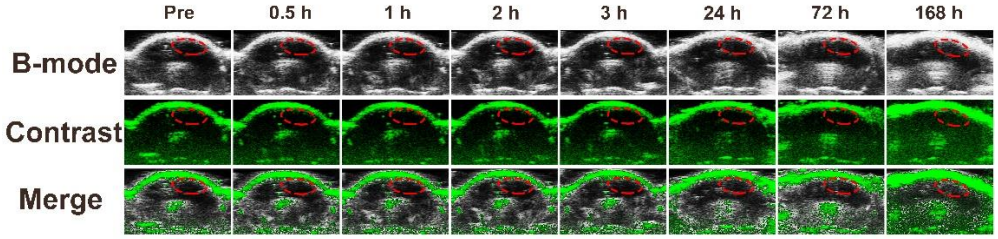

Figure S12. *In vivo* ultrasound images of mice brain before (0 h) and after PMVs injection at different time points (0.5, 1, 2, 3, 24, 72, 168 h).

Table S1. LC-MS/MS total proteins number analysis of PLT, PMVs and PNBs samples.

	PLT	PMVs	PNBs
Total proteins number	2220±21	1971±13	2015±12

Table S2. Identified vascular adhesion, thrombus inhibition, and immunoglobulin related receptor proteins for PLT, PMVs and PNBs.

Samples / Protein description	Intensity (a.u.)		
	PLT	PMVs	PNBs
Integrin beta-1	$(1.63 \pm 0.09) \times 10^{9}$	$(1.23 \pm 0.06) \times 10^{10}$	$(1.75 \pm 0.12) \times 10^{10}$
Integrin beta-3	$(3.43 \pm 0.19) \times 10^{10}$	$(1.27 \pm 0.02) \times 10^{11}$	$(2.01 \pm 0.15) \times 10^{11}$
GPVI (collagen binding)	$(1.67 \pm 0.29) \times 10^{9}$	$(9.56 \pm 1.13) \times 10^{8}$	$(7.38 \pm 1.14) \times 10^{8}$
GPIIb-IX-V complex (vWF binding)	$(6.57 \pm 0.19) \times 10^{10}$	$(1.88 \pm 0.08) \times 10^{11}$	$(2.27 \pm 0.15) \times 10^{11}$
CD47 (immunomodulatory)	$(5.83 \pm 0.38) \times 10^{8}$	$(3.55 \pm 0.03) \times 10^{9}$	$(2.76 \pm 0.22) \times 10^{9}$
CD55 (immunomodulatory)	$(3.33 \pm 0.94) \times 10^{7}$	$(4.45 \pm 0.65) \times 10^{8}$	$(5.04 \pm 0.52) \times 10^{8}$
CD59 (immunomodulatory)	$(4.03 \pm 0.94) \times 10^{7}$	$(3.26 \pm 0.11) \times 10^{8}$	$(5.03 \pm 1.25) \times 10^{8}$
Platelet endothelial cell adhesion molecule (PECAM-1/CD31) (integrin activation for thrombosis/hemostasis)	$(1.38 \pm 0.10) \times 10^{9}$	$(1.13 \pm 0.13) \times 10^{10}$	$(1.20 \pm 0.08) \times 10^{10}$