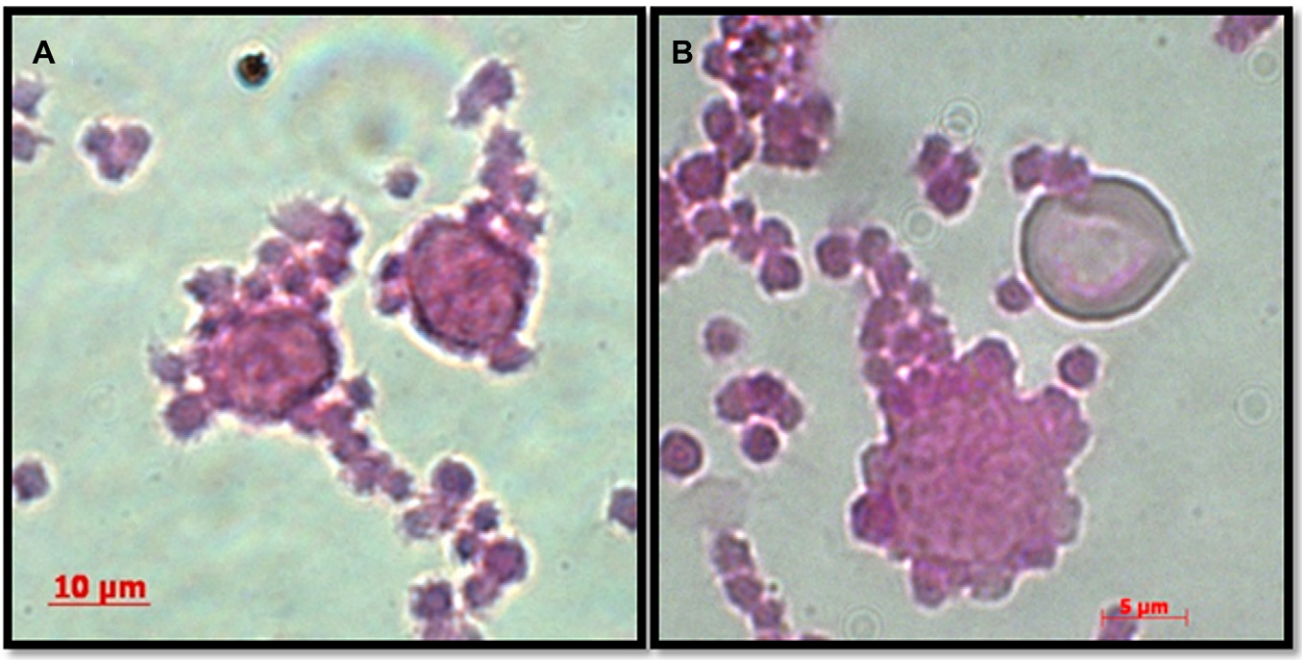
Supplementary materials and methods

Staining of megakaryocytic cells

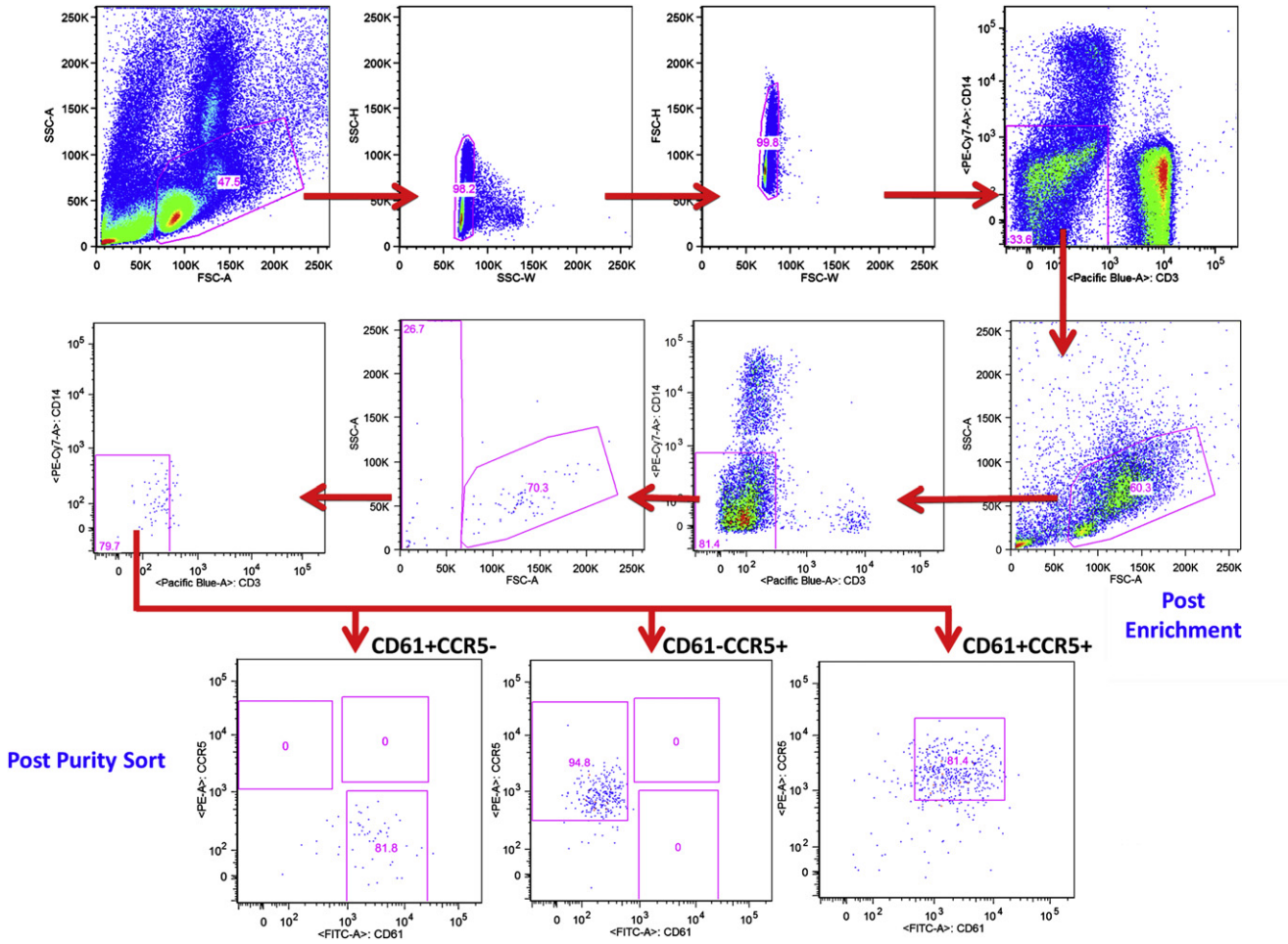
BM smears were subjected to periodic acid-Schiff staining with the PAS Kit (Polysciences Inc., Warrington, PA, USA) performed according to manufacturer's suggested protocol. For the double immunohistochemical staining for DENV antigen and CD41a, megakaryocytes were collected from BM using a method described by Wilde et al. [20]. Briefly, 1 mL BM suspension was passed via gravity through an assembled syringe filter holder containing a 5-μm pore diameter nucleopore polycarbonate membrane (Fisher Scientific, Pittsburgh, PA, USA). After two washes with PBS, the membrane was removed and left to dry thoroughly. Thereafter, cells on the membrane were fixed with acetone/methanol (50:50) for 90 seconds, treated with 0.6% H_2O_2 for 30 minutes to block endogenous peroxidase, and then blocked for 30 minutes with 10% human AB serum. After two washes with PBS, the samples were treated with avidin and biotin solutions according to the manufacturer's instructions provided in the avidin/biotin blocking kit (Vector) and then incubated with mouse anti-E monoclonal antibody (1:100; clone 3H5) or its isotype-matched control at 4°C overnight. The samples were washed three times with PBS and incubated with biotinylated horse anti-mouse immunoglobulins (Vector) at room temperature for 30 minutes followed by three washes with the same buffer. The samples were then incubated for 30 minutes each with Vectastain ABC reagent and 3-amino-9-ethylcarbazole (Vector) for the development of peroxidase signal. The samples were then washed three times with PBS, incubated with 10% normal mouse serum for 30 minutes, and then labeled with fluorescein isothiocyanate−conjugated mouse anti-human CD41a antibody (1:50; BD Biosciences) for 1 hour. After washing with PBS, the samples were incubated with rabbit anti–fluorescein isothiocyanate antibody conjugated to alkaline phosphatase (1:250; Sigma Aldrich, St Louis, MO, USA) and the signal was developed with the Vector blue alkaline phosphatase substrate kit III in the presence of levamisole solution (Vector), an inhibitor of endogenous alkaline phosphatases. The resulting images were captured with a Zeiss microscope equipped with Axis 5 digital camera.

Measurement of nonstructural protein 1 concentration in supernatant fluids of cocultures of flow cytometry–assisted sorted cells with Vero cells

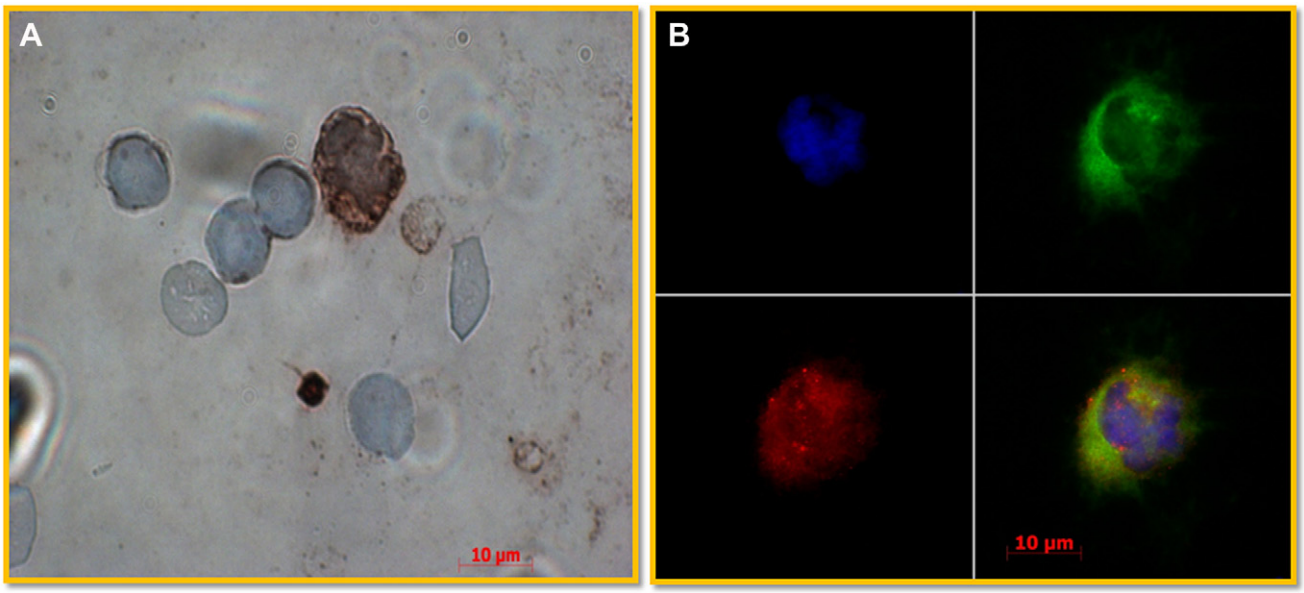
Subsets of BM–derived $CD61^-CCR5^+$, $CD61^+CCR5^+$, and $CD61^+CCR5^-$ cells were isolated using flow cytometry and cocultured with Vero cells and supernatant fluids of the coculture were collected daily for 7 days and stored at -80°C until assay. Standard enzyme-linked immunosorbent assay was set up to quantify nonstructural protein 1 antigen in the collected supernatant fluid by using purified nonstructural protein 1 antigen (CTK Biotech, Inc, San Diego, CA, USA) as a standard control.



Supplementary Figure E1. Two representative photographs of cells (**A**, **B**) showing platelet-like vesicles budding from the cell surface in freshly prepared blood smears from dengue-infected rhesus monkeys. Freshly prepared peripheral blood mononuclear cells were applied onto slides and subjected to periodic acid-Schiff assay.



Supplementary Figure E2. Flow cytometric strategy to sort cells expressing the cells surface markers $CD61^+$ and/or $CCR5^+$. BM aspirates were pooled and subjected to antibody staining and a two-step fluorescence-activated cell sorting was applied as described in the Materials and Methods. Figure at the bottom shows the purity of the sorted cells upon reanalysis.



Supplementary Figure E3. Representative photomicrograph depicting the immunostaining profile of post flow cytometry obtained enriched subset of cells that confirms the presence of dengue antigen within $CD61^{+}$ cells. Cells collected from post enrichment were subjected to cytospin onto slides and subjected to immunostaining. (**A**) Immunohistochemical staining depicts dengue antigen (brown) and nucleus (light blue). (**B**) Immunofluorescence staining depicts CD61 (green), a megakaryocytic marker, dengue antigen (red) and nucleus, 4′,6-diamidino-2-phenylindole (blue).

Supplementary Table E1. Viral load in the BM of DENV-infected rhesus monkeys*

	Days PI	1	2	3	4	5	6	7
Platelets	RM#1	2.65×10^2			5.12×10^5		ND	
	RM#2		4.92×10^5			1.4×10^5	ND	
	RM#3			2.7×10^5				ND
Plasma	RM#1	ND			1.35×10^2		ND	
	RM#2		1.88×10^2			5.27×10^3	3.82×10^2	
	RM#3			7.2×10^2				ND

ND = not detectable; PI = post infection.
*Viral load was determined in platelets or plasma fraction of the bone marrow by qRT-PCR as previously described [16].