Supplemental Methods

Compounds and Reagents

Naturally derived caraphenol A and α-viniferin were purchased (caraphenol A CAT# 354553-35-8, α-Viniferin CAT# 78690-98-9, BOC Sciences, Shirley, NY) and purified by preparative reverse-phase HPLC (CH_3CN/H_2O-0.07% TFA 10:90 to 90:10 over 30 min, 3 mL/min, R_t = 21.4 min) to provide pure caraphenol A and α-viniferin, possessing NMR spectral data consistent with prior reports. Cytokines IL-3 (CAT# 10779-594), IL-6 (CAT# 200-06), TPO (CAT# 300-18), SCF (CAT# 300-25), and Flt3-L (CAT# 300-19) were all ordered from Peprotech. BIT 9500 was purchased from Stem Cell Technologies (CAT# 09500).

HPLC purification of compounds

HPLC was conducted using a Waters 600 pump/controller, a Waters 996 photodiode array detector, and a Cosmosil 5C18-AR-II column. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker Avance III HD 600 MHz spectrometer equipped with either a 5 mm QCI or 5 mm CPDCH probe.

Lentiviral Vector

Transgene plasmid and accessory packaging plasmids were transfected with calcium-phosphate onto minimal passage 293T cells. 36 h after transfection, supernatant was collected and concentrated by ultracentrifugation on a sucrose gradient. MOI was calculated by a dilution series on HEK 293T lines and analysis of EGFP expression by flow cytometry. IDLV was constructed, packaged, and used as previously reported[1].

LV Transduction

All HeLa cells were grown in DMEM (Corning CAT# 15-013-CV) containing 10% FBS (Omega Scientific, CAT# FB-01), 1% Pen/Strep (Invitrogen CAT#15140122), and 1% L-Glutamine (Invitrogen CAT#25030081). HeLa cells were split at $2x10^4$ cells/well of a 48-well plate and grown overnight. Cells were incubated over 8 h with LV and indicated compounds, after which, both were removed and the cells were cultured for 5 days before flow cytometry analysis. Cells were removed with trypsin and neutralized with FACs buffer (PBS+2%FBS), pelleted at 300xg, and resuspended in FACs buffer. Flow cytometry was performed on a BD LSR-II flow cytometer.

$CD34^+$ Cell Isolation and Use

$CD34^+$ cells were isolated under approved institutional protocol (information and approval is available upon request) and in accordance with the Declaration of Helsinki. mPBs $CD34^+$ cells were grown in SCGM (CellGenix CAT# 20802-0500), while UCB $CD34^+$ cells were grown in IMDM, with supplements, as previously reported[2]. mPBs $CD34^+$ cells were pre-stimulated for 48 h with 0.1µg/mL TPO, 0.1µg/mL SCF, and 0.1µg/mL Flt3-L before LV addition. UCB $CD34^+$ cells were pre-stimulated for 24 h as previously reported[2]. During LV transduction, mPB and UBC $CD34^+$ cells were cultured under identical conditions as pre-stimulation, with the addition of 4µg/mL Polybrene. $CD34^+$ cells were incubated for 4 h with either DMSO vehicle control, caraphenol A, PGE-2 or rapamycin, after which they were transduced with LV for another 20 h in the absence or presence of selected LV transduction enhancers. After LV transduction, washed $CD34^+$ cells were cultured with: 0.1µg/mL TPO, 0.1µg/mL SCF, 0.1µg/mL FLT-3, 0.06µg/mL IL-3, 0.06µg/mL IL-6 for mPB cells, and 0.1µg/mL SCF, 0.05µg/mL IL-3, 0.05µg/mL IL-6, and 10%FBS for UCB cells. All $CD34^+$ cells were cultured for 7-14 days and then analyzed by flow cytometry (BD LSR-II). For AAV experiments, UCB cells were isolated, cultured and pre-stimulated as above. AAV was added for 24 hours before removal from culture. AAV mCherry was a kind gift of Prashant Mali (University of California, San Diego).

Lentiviral Vector Blam-Vpr Assay

UCB- or mPB-derived $CD34^+$ cells were pre-treated for 4 h with DMSO or caraphenol A, then transduced with pRRLSIN-MND-NGFR[2] carrying the -Vpr protein (MOI=15) for an additional 6 h in the presence of DMSO or caraphenol A. Cells were then washed and resuspended in 125 µl loading medium (IMDM containing 20% BIT9500 or SCGM, no antibiotics). Substrate loading and cellular analyses were completed, as previously reported[2]. The Vpr kinetic assay was developed from methods previously described[3], with a slight modification transitioning cells to 4°C at indicated timepoints; compounds and vector were then washed out and samples were loaded as above for 8 h at 12°C, before processing for flow cytometry.

$CD34^+$ Cell Viability, Proliferation, and Colony Forming Unit Assessment

UCB $CD34^+$ cells were pre-treated for 4 h with the indicated dose of compounds before transduction with LV for 20 h. Cells were then washed and seeded, in duplicate or triplicate, as described, for 14 days. At the indicated times aliquots of LV transduced cells were analyzed for viability, as previously reported[2]. Colony-forming unit (CFU) assessment of $CD34^+$ progenitor cell differentiation was completed as previously described[4].

Ectopic IFITM Expressing Cell Lines

pQCXIP-FLAG-IFITM1, -IFITM2, -IFITM3, and -IFITM3 Δ17-20 (gifts from C. Liang), were transfected into 293T cells with Mirus TransIT-LT1. Stably expressing cells were created following selection with puromycin for two weeks and analyzed by Western Blot analyses for protein expression.

TZM-bl IFITM3 KO Cell Line

IFITM3 KO TZM-bl cells[5] were created by transfection with a set of plasmids encoding Cas9, three *IFITM3*-specific guide RNAs (sc-403281, Santa Cruz Biotechnology), and a set of three plasmids providing templates for homology-directed repair (sc-403281-HDR, Santa Cruz Biotechnology). A population of modified cells was selected following puromycin treatment for three weeks.

VCN Analyses

VCN was established through quantitative PCR from the genomic DNA of total cell populations. Cells were collected and processed by the Qiagen DNeasy Blood and Tissue Kit (CAT# 69506). qPCR for LV targeted the late product U5Ψ with primers MH531 and MH352 with probe LRT-P, while early product RU5 used primers hRU5-F2 and hRU5-R with probe hRU5-P[6]. Genomic loading was standardized with Taqman RNaseP (CAT# 4401631). A Roche LightCycler 480 was used for genomic product amplification and analyses.

Quantitative IFITM3 RT-PCR

$1x10^5$ HeLa cells were seeded one day prior to treatment and incubated with compound for indicated time periods. Cells were collected and total mRNA was isolated using RNeasy Plus Mini Kit (Qiagen, CAT#74134) according to manufacturer protocols. Extracted RNA was reverse transcribed using iScript cDNA Synthesis Kit (Bio-Rad, CAT#1708891). Expression analysis was undertaken using TaqMan probes from Integrated DNA Technologies that specifically amplified the coding regions of endogenous human IFITM3. Primers sequence for IFITM3: 5'IFITM3-TCACACTGTCCAAACCTTCTTCT, 3'IFITM3 - GCCCCCAGCACAGCCACCTCG, Probe IFITM3 CAGCGGCCAGCCCCCCAACTATG. qPCR for was conducted on a Roche LightCycler 480 was used for product amplification and analyses. qPCR was run for 40 cycles to extract raw Ct values and then ΔCt were calculated, comparing Ct values of the reference gene GAPDH.

Mouse Transplantation and Analysis

For human $CD34^+$ HSPC transplantation studies, 4-6 week old female mice were randomly assigned to experimental groups and irradiated at 240 cGy using a cesium source. $3x10^5$ UCB $CD34^+$ LV transduced cells per mouse were injected retro-orbitally and the remaining cells were cultured *ex vivo* for transgene expression analyses 7 and 14-days post-transduction. Peripheral blood was sampled every 3-5 weeks after an initial 6-7 week engraftment period, with red cells removal before flow cytometry analyses, as previously reported[2]. Mice were sacrificed at 22 weeks (terminal) and harvested for peripheral blood, bone marrow, and spleen. Engraftment was determined with BD BioSciences antibodies BUV395-mCD45 (CAT# 564279) and APC-hCD45 (CAT# 555485). Lineage was determined with antibodies PerCP-Cy5.5-hCD3 (CAT# 560835), V450-hCD19 (CAT# 560353), and PE-hCD33 (CAT# 555450). Gating was established using fluorescence minus one controls (FMOC) and EGFP expression was gated using human $CD34^+$ engrafted but non-transduced mice. All flow cytometry was performed on a BD LSR-II.

Western Blotting

$5x10^4$ LV transduced or control HeLa cells were lysed directly in wells on ice with 75 µL of RIPA buffer (CAT# 89900) plus Proteinase Inhibitor (CAT# 87785). Lysates were measured for protein concentration with Pierce BCA Protein Assay Kit (CAT# 23227) and normalized. Samples were processed with LDS Sample Buffer (CAT# B0007) and Sample Reducing Agent (CAT# B0004). Lysates were run on 4-12% Bis-Tris Bolt Precast Gel from Invitrogen (Cat# NW04120BOX) and transferred to an activated PVDF FL membrane. Membranes were incubated with dilutions of the following primary antibodies: anti-IFITM2 (Proteintech, 66137-1-Ig), anti-IFITM3 (Abcam, ab109429), anti-IFITM2/3 (Proteintech, 66081-1-Ig). Membranes were then incubated with an HRP secondary and exposed to film or imaged using the Azure c600 imaging system (Azure

Biosystems). Western blot signal quantification was conducted using AzureSpot analysis software (Azure Biosystems).

Integration Site Analysis

Processing of gDNA to amplify integration loci included modified genomic sequencing (MGS)-PCR [7], followed by Illumina paired-end Miseq next generation sequencing. Integration sites were identified by a method similar to that described by Hocum *et al.* 2015 [8] using a published pipeline which includes custom scripts and is available upon request [9]. Additional information is provided in the Supplemental Methods section.

The *Homo sapiens* reference genome (GRCh38/hg38, GCA_000001405.15, Dec. 2013) provided by the Genome Reference Consortium was downloaded from the UCSC genome browser (http://genome.ucsc.edu/). Resulting files were parsed for multiple possible alignments for each sequence read, such that any sequence read with a secondary alignment with percent identity up to 95% of the best alignment was discarded. Sequence reads were then grouped based on their genomic alignment positions and orientation (sense (+) vs. antisense (-)). Any alignments within 5 base pairs of one another with identical orientations were considered to originate from the same integration event; the genomic position with the greatest number of contributing sequence reads was defined as the integration locus. A custom Python script was used to localize integration sites to genomic features using Refseq gene lists available from the UCSC genome browser and to known oncogenes using COSMIC: catalog of somatic mutations in cancer (https://cancer.sanger.ac.uk/cosmic).

IFITM Intracellular Analysis

Cells were fixed/permeabilized with Cytofix/CytoPerm reagent from BD (CAT#554714) for 20 min and washed in Perm/Wash buffer from BD (CAT#554723). Cells were pelleted and resuspended in anti-IFITM2/3 antibody diluted in Perm/Wash buffer, incubated at room temperature for 30 min, and washed in Perm/Wash buffer. Cells were acquired and analyzed on a LSRFortessa (BD).

Confocal Microscopy of HeLa and TZM-bl Cells

1×10^5 HeLa or TZM-bl cells were plated on poly-L lysine coated coverslips, 1 day after plating, cells were pre-treated for 4 h with the indicated doses of compounds, before addition of LV at a MOI of 15. Cells were treated with LV for 30 min or 2 h in 48 well plates, before washing 3 times in 2%FBS + PBS buffer to remove attached surface bound LV**.** Cells were then fixed in 3.7% confocal grade paraformaldehyde (ThermoFisher Scientific, CAT# 50-980-487,) for 8 min and washed 3 times with confocal wash buffer (PBS + 0.1% saponin and 1% BSA). After fixing, cells were treated overnight at 4 °C with mouse anti-IFITM2/3, rabbit anti-LAMP1 Antibody (ThermoFisher Scientific, CAT# PA1-654A) both diluted 1:400 and goat anti-HIV p24 (Abcam, CAT# ab53841) diluted 1:100 in confocal wash buffer. After incubation, slides were again washed 3 times with wash buffer and incubated for 1hr with Alexa Fluor® 488 donkey anti-mouse antibody (ThermoFisher Scientific, CAT# A-21202), Alexa Fluor® 568 donkey anti-goat antibody (ThermoFisher Scientific, CAT# A-11057) and Alexa Fluor® 647 Donkey anti-rabbit IgG (minimal x-reactivity) Antibody (BioLegend, CAT# 406414) at 1:200 at room temperature. After antibody treatment, slides were again washed 3 times with wash buffer then treated for 30 min with Hoechst 33342 nuclei stain (ThermoFisher Scientific, H3570) at 1:2000 dilution, washed again 3 times and mounted on slides for 3 d with ProlongGold Antifade Reagent (ThermoFisher, CAT# P36930). Confocal microscopy analysis of vector and endosomal markers was performed using a Zeiss 710 laser scanning confocal microscope at 64× oil-immersion magnification at a 2-fold digital

zoom with a 1024×1024 field size. For establishing voltage and aperture for confocal microscopy, snap images were captured for each channel using control slides treated only with secondary antibody or no vector added. Z-stack depth was set using the Hoechst channel, with images collected in 16 slice intervals, 0.2μm per image, collected at ~1 image per min. At least 50 cells total were imaged for each condition. Z-stacks were analyzed with Imaris Software (Bitplane, Zurich, Switzerland) using the ImarisCell analysis module, quantifying number and intensity of IFITM2/3 and LAMP-1$^+$ vesicles. Cell boundaries were identified using the 647 channel, with thresholding set by untreated or secondary antibody treated control images. Statistical analysis of vesicles number and average staining intensity was conducted using GraphPad Prism 7 software (GraphPad, San Diego, CA), and evaluated with the Kruskal-Wallis test using Dunn's multiple comparison correction.

Confocal Microscopy of mPB CD34$^+$ Cells

Mobilized peripheral blood CD34$^+$ cells were thawed and pre-stimulated, then plated in 48 well plates and treated with compound as described above. 1×10^5 CD34+ cells were transduced per timepoint and compound condition. After washing, cells were spun via cytospin onto positively charged confocal grade microscopy slides at 450xg for 5 min. After cytospin, cells were fixed, permeabilized and stained as above for HeLa and TZM-bl cells.

Supplementary Figure Legends:

Supplementary Figure 1. Characterization of synthetic versus HPLC purified caraphenol A and effect of compounds on HEK 293T transduction and HeLa cell growth. (**a**) Wild-type HEK293T cells were transduced with pRRLSIN-MND-EGFP (termed LV) at indicated multiplicity of infection (MOI), in the presence of DMSO (diluent control, blue circles) or indicated concentrations of resveratrol (orange circles), α-viniferin (purple circles), (rapamycin, red circles) or caraphenol A (green circles) over 8 h, before removal of compound and LV and then *ex vivo* culture. Cells were analyzed 5 days later by flow cytometry for EGFP expression (n=3 independent experiments). Data are shown as linear plots (mean ± s.d.), all results were non-significant by two-tailed Student's t-test, comparing percent EGFP expression in caraphenol A and DMSO treated cells. (**b**) HeLa cells (n=2 independent experiments, 4 cultures) were treated with either resveratrol (30μM), caraphenol A (30μM, green) or α-viniferin (30μM, purple) for the indicated time periods before compound removal and continued culture for 3 days. Cell metabolic activity was measured by the MTT assay with 570nm absorbance values normalized to cells treated with resveratrol set as 1.0. Data presented as linear graphs (mean ± s.d). (**c**) Umbilical cord blood-derived (UCB) $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) (n=2 donors) were pre-stimulated for 24 h before 4-h pre-treatment with either DMSO (0.06%) or caraphenol A (30μM) derived from either synthesis[10] or HPLC purified naturally derived compound. LV was then added for 20 h before compound and LV removal, culture, and analysis by flow cytometry for EGFP expression 7 days later. Data presented as bar graph (mean ± s.d). DMSO versus synthetic **P= 0.0037, DMSO versus purified ***P= 0.0006, synthetic versus purified n.s.= not significant by Student's two tailed t-test. (**c**) Commercial caraphenol A was purified by preparative reverse-phase HPLC (CH_3CN/H_2O-0.07% TFA 10:90 to 90:10 over 30 min, 3 mL/min, R_t = 21.4 min) to provide pure caraphenol A, which displayed spectral data consistent with prior reports[10]. ^1H NMR (acetone-d_6, 600 MHz) δ 7.27–7.21 (m, 4H), 7.07–7.03 (m, 2H), 6.92 (d, J = 2.0 Hz, 1H), 6.82–

6.77 (m, 3H), 6.77–6.73 (m, 2H), 6.71–6.68 (m, 2H), 6.54 (dd, *J* = 1.9, 1.1 Hz, 1H), 6.49 (dd, *J* = 2.2, 0.5 Hz, 1H), 6.31 (d, *J* = 2.2 Hz, 1H), 6.24 (dd, *J* = 2.1, 0.7 Hz, 1H), 5.91 (s, 2H), 4.85 (s, 1H), 4.33 (s, 1H). (**d**) Caraphenol A ^{13}C NMR (acetone-d_6, 150 MHz) δ 163.6, 160.6, 160.0, 159.2, 158.4, 158.2, 158.1, 157.1, 155.3, 149.7, 141.1, 139.8, 135.4, 133.6, 132.8, 128.4, 128.3, 127.4, 123.0, 122.8, 120.7, 119.2, 116.3, 116.1, 115.9, 114.5, 109.8, 108.8, 108.7, 98.5, 97.6, 96.4, 95.2, 88.0, 54.1, 46.0 (**e**) Commercial α-viniferin was purified by preparative reverse-phase HPLC (CH_3CN/H_2O-0.07% TFA 10:90 to 90:10 over 30 min, 3 mL/min, R_t = 25.6 min) to provide pure α-viniferin, which displayed spectral data consistent with prior reports [11]. ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.24–7.19 (m, 2H), 7.06–6.99 (m, 4H), 6.81–6.75 (m, 4H), 6.74–6.68 (m, 3H) 6.59 (d, *J* = 2.1 Hz, 1H), 6.26–6.21 (m, 3H), 6.07 (s, 1H), 5.99 (dd, *J* = 2.2, 0.6 Hz, 1H), 5.95 (d, *J* = 9.9 Hz, 1H), 4.91 (d, *J* = 6.3 Hz, 1H), 4.71 (dd, *J* = 9.9, 0.9 Hz, 1H), 4.62 (d, *J* = 6.2 Hz, 1H) 3.96 (s, 1H). **(f)** α-viniferin ^{13}C NMR (acetone-d_6, 150 MHz) δ 161.8, 161.7, 160.9, 160.8, 159.4, 159.4, 158.5, 158.3, 157.9, 141.3, 139.8, 138.8, 132.6, 132.3, 132.1, 128.7, 128.3, 128.2, 120.9, 119.8, 118.9, 116.1, 115.7, 108.6, 106.3, 105.8, 98.1, 97.0, 96.6, 95.7, 90.0, 86.5, 55.7, 52.9, 46.5.

Supplementary Table 1: Comparison of spectral data for synthetic versus naturally-derived, HPLC purified caraphenol A and α-viniferin.

Supplementary Figure 2. Caraphenol A does not effect CD34$^+$ HSPC viability, proliferation or differentiation capacity of progenitor cells, and has additive effects with other transduction enhancing compounds. (**a**) Effects of caraphenol A (10μM open green circles or 30μM closed green circles), and rapamycin (20μg/mL, red circles) on proliferation of UCB-derived CD34$^+$ HSPCs (n=3 donors). Cells were pre-stimulated for 24 h then incubated with the indicated compound and LV for 24 h before removal and continued culture for 7 days. Cells were counted at indicated timepoints and data presented as dot plots (mean ± s.d). (**b**) mPB CD34$^+$ HSPCs

(n=5 donors for 30 μM caraphenol A, n=2 or 3 donors for other concentrations) were pre-stimulated for 48 h, then cultured with indicated concentrations of compounds and LV as above. Cell viability was measured as percent trypan blue excluding cells at 7 days and reported as bar graphs (mean ± s.d). Transduction was measured as percent EGFP+ cells after 7 days and reported as dot plots (mean ± s.d) (**c**) UCB $CD34^+$ HSPCs (n=2 donors, 3 replicates per condition) were mock treated or with DMSO as above then transduced with LV as above. Cells were then assessed for progenitor proliferation capacity by colony-forming unit assay, evaluating plating efficiency as a percent of input cells and (**d**) colony type (CFU-GM = granulocyte-macrophage progenitor, CFU-GEMM = granulocyte, erythrocyte, monocyte, megakaryocyte progenitor, BFU-E = erythroid burst-forming unit) 14 days later. Data presented as bar graphs (mean ± s.d). (**e**) Human UCB-derived $CD34^+$ HSPCs (n=2 donors) were pre-stimulated and treated with either DMSO (0.06%, blue), caraphenol A (30μM, green) prostaglandin E2 (PGE2, 10μM, grey), rapamycin (20μg/mL, red), PGE-2 + caraphenol A (grey diamonds, green border), rapamycin + caraphenol A (red circles, green border) or PGE-2 + rapamycin (grey boxes, red border) for 4 h, before LV (MOI 8) addition for 20 h, before removal and analysis by flow cytometry 7 days later for EGFP expression. Data presented as dot plot (mean ± s.d), **P<0.0021, ****P<0.0001, n.s.=not significant by ordinary one-way ANOVA with Dunnets's multiple comparison test, comparing to DMSO. (**f**) Viability of cells co-treated as above with indicated compounds were analyzed for cell viability at indicated timepoints using trypan blue exclusion.

Supplementary Figure 3. Caraphenol A improves marking of human cell lineages and does not alter lineage development in humanized mice. (**a**) Gating strategy for flow cytometric analysis of caraphenol A and DMSO treated human cell populations in bone marrow of engrafted NSG mice. (**b**) Percent human $CD45^+$ cells in peripheral blood at indicated timepoints after treatment for MOI 10 (top, closed circles) and MOI 25 (bottom, open circles) cohorts, comparing

short term engraftment ability of DMSO (blue circles) and caraphenol A (green circles) UCB $CD34^+$ cells. Data presented as dot plots (mean ± s.d) n.s.= not significant by two-tailed Mann-Whitney test. (**c**) Percent human $CD45^+EGFP^+$ cells in CD3+ T cell (left), $CD19^+$ B cell (middle) and $CD33^+$ myeloid (right) subsets in bone marrow of UCB $CD34^+$ cell engrafted NSG mice (n=8 mice per treatment, 16 mice per MOI) at terminal timepoint, comparing $EGFP^+$ expression in caraphenol A and DMSO mice. Data presented as dot plots (mean ± s.d). $CD3^+$ MOI 10 (closed circles) *P= 0.014, MOI 25 (open circles) n.s. = not significant, $CD19^+$ MOI 10 (closed circles) **P= 0.0059, MOI 25 (open circles) n.s.=not significant, $CD33^+$ MOI 10 (closed circles) **P= 0.0042, MOI 25 (open circles) n.s.=not significant by two-tailed Mann-Whitney test. (**d**) Percent of human $CD3^+$, $CD19^+$ and $CD33^+$ subsets in bone marrow measured per total human $CD45^+$ leukocytes of caraphenol A and DMSO treated humanized mice. Data presented as dot plots (mean ± s.d), MOI 10 (closed circles), MOI 25 (open circles) n.s.= not significant by two-tailed Mann-Whitney test.

Supplementary Figure 4. Caraphenol A improves gene delivery efficiency and does not alter lineage development in serially transplanted NSG mice. (**a**) UCB-derived $CD34^+$ HSPCs were treated with caraphenol A (green squares) or DMSO (blue circles) as above and transduced with LV at MOI 25, before engraftment in lethally irradiated NSG mice. Cells were analyzed for EGFP expression by flow cytometry after 7 days of *ex vivo* culture (left) and after 22 weeks of engraftment from mouse bone marrow (right). (**b**) Comparison of donor engraftment in bone marrow at terminal timepoint, as measured by total proportion of leukocytes that were $mCD45^-$ $huCD45^+$. (**c**) Percent of human $CD19^+$, $CD33^+$ and $CD3^+$ subsets in bone marrow measured per total human $CD45^+$ leukocytes of caraphenol A and DMSO treated humanized mice and (**d**) percent of $EGFP^+$ cells within each lineage subset. (**e**) After isolation of total human $CD34^+$ cells from bone marrow of primary engraftment experiment above, $1x10^5$ cells were engrafted in secondary transplant lethally irradiated NSG mouse recipients. The highest, middle and lowest

EGFP+ expressing individuals from caraphenol A (green circles) and DMSO (blue circles) treated groups were chosen for secondary engraftment. Secondarily engrafted mice were sacrificed at 12 weeks and analyzed for percent human cell engraftment as above, along with (**f**) human cell lineage and (**g**) EGFP marking of different lineages. All data presented as dot plots (mean ± s.d.). n.s.=not significant, **P<0.0042, ***P<0.0006 by two-tailed Mann-Whitney test.

Supplementary Figure 5. Caraphenol A treatment improves VSV-G LV post-entry endosomal release into the cytoplasm and does not enhance measles LV or AAV vector entry. (**a**) Human UCB-derived $CD34^+$ HSPCs (n=3 donors) were pre-stimulated and treated with caraphenol A (30μM) or DMSO (0.06%) for 24 h before analysis of cell-surface expressed LDL receptor (LDLR) mean fluorescence intensity (left) and percent total expression (right) by flow cytometry. Data presented as bar graph (mean ± s.d), n.s.=not significant by two-tailed Student's t-test. (**b**) Human UCB $CD34^+$ HSPCs (n=3 donors) were pre-stimulated and pre-treated with caraphenol A (30μM) or DMSO (0.06%) for four h before addition of measles hemagglutinin and fusion glycoprotein (MOI 1) or vesicular stomatitis virus glycoprotein (VSV-G, MOI 8) pseudotyped LV for 20 h. After LV and compound removal, cells were cultured for 7 days before analysis for EGFP expression by flow cytometry. Data presented as bar graph (mean ± s.d), ***P= 0.0015, n.s.=not significant by two-tailed Student's t-test. (**c**) Human UCB $CD34^+$ cells were isolated and pre-treated with caraphenol A, DMSO or mock treated as described above, before addition of either $1x10^4$ virions/cell (open bars) or $1x10^5$ virions/cell (solid bars) of an AAV6 serotype vector carrying mCherry. Cells were analyzed by flow cytometry 3 days later (n=2 donors). Data presented as bar graph (mean ± s.d), n.s.=not significant by two-tailed Student's t-test. (**d**) Human mPB $CD34^+$ cells (n=3 donors) were transduced in the presence of DMSO (0.06%, blue circles), caraphenol A (30μM, green circles) or resveratrol (30μM) with pRRLSIN-MND-NGFR, MOI I5, carrying the BLAM-Vpr protein. After a 6-h transduction, cells were loaded with the BLAM

substrate CCF2-AM, and cytoplasmic presence was quantified by flow cytometric detection of cells exhibiting cleaved CCF2. Transduction was measured by NGFR expression 7 days later. Data presented as dot plots (mean ± s.d). Fusion **P=0.0058, transduction *P=0.033 by two-tailed Student's t-test. (**e**) Human mPB $CD34^+$ cells were transduced in the presence of DMSO (0.06%) or caraphenol A (30μM) using above BLAM-Vpr containing vector, then transferred to 4°C at indicated timepoints, before loading with BLAM substrate CCF2-AM at 12°C overnight. LV-EGFP cytoplasmic fusion was quantified by flow cytometric analysis for cleaved CCF2. Data presented as linear plots (mean± s.d). Combination figure ****P<0.0001, slopes of linear regression significantly different. (**f**) Pre-stimulated human UCB $CD34^+$ cells (n=2 donors) were transduced with LV (MOI 25) in the presence of DMSO (0.06%, blue circles) and caraphenol A (30 μM, green circles) and strong-stop DNA (Early RT) or (**g**) full-length DNA (Late RT) HIV-1 reverse-transcription products were quantified by qPCR. Data reported as fold change in LV copies per cell relative to DMSO and presented as box plots (mean, min-to-max). (**h**) The ratios between Late RT to Early RT reverse-transcription products were calculated for each donor. **P<0.0055 from a parametric 2-tailed paired Student t-test. LTR, long terminal repeat.

Supplementary Figure 6. IFITM2 & 3 are expressed in HeLa and mPB $CD34^+$ cells and IFITM proteins are downregulated by caraphenol A but not PGE-2. (**a**) SDS-PAGE and western blot analysis of 293T whole cell lysate from cells transfected with pQCXIP-FLAG-IFITM1, -IFITM2, or -IFITM3 for 48 h. Immunoblotting was performed with anti-IFITM2, anti-IFITM3, anti-IFITM2/3, and anti-FLAG antibodies showing similar amounts of IFITMs. (**b**) Flow cytometric analysis of pQCXIP-FLAG-IFITM1, -IFITM2, or -IFITM3 stably transfected 293T cells. Cells were immunostained with anti-FLAG antibodies. Data presented as univariate histograms of protein expression from one experiment, representative of 3 replicate experiments. (**c**) SDS-PAGE and western blot analysis of cell lysates generated from HeLa cells treated with caraphenol A (30μM)

for the indicated time period. Immunoblotting was performed with α-IFITM2/3 specific antibody and α-GAPDH as a loading control. Numbers indicate location and size (kD) of protein standards in ladder. Image provided is a representative blot of 2 experiments. (**d**) HeLa cells (n=3 independent experiments) were treated with DMSO (0.06%, blue), resveratrol (30μM, orange), caraphenol A (30μM, green) and rapamycin (10μg/mL, red) for indicated time and cells were either immediately harvested or washed and cultured for an additional 16 hours, before analysis for IFITM3 and GAPDH transcription by quantitative RT-PCR. (**e**) SDS-PAGE and western blot analysis of cell lysates generated from HeLa cells treated with PGE-2 (10μM) for the indicated time period. Immunoblotting was performed with α-IFITM3 specific antibody and α-GAPDH as a loading control. Numbers indicate location and size (kD) of protein standards in ladder. Image provided is a representative blot of 2 experiments. (**f**) Representative plots and (**g**) summary of (n=3) experiments of pQCXIP-FLAG-IFITM1, -IFITM2, or -IFITM3 stably transfected 293T cells treated for 4 hours with DMSO (0.06%, red), caraphenol A (30μM, green or 50μM, orange) or rapamycin (20μg/mL, blue) and then intracellularly stained by anti-FLAG antibodies. (**h**) SDS-PAGE and western blot analysis of TZM-bl (WT and *IFITM3* KO) whole cell lysates. Immunoblotting was performed with anti-IFITM2 and anti-IFITM3 antibodies. (**i**) TZM-bl wild type cells were treated with either DMSO, rapamycin or caraphenol A for 4 h either alone or in the presence of bafilomycinA1. Whole cell lysate was then evaluated by SDS-PAGE and western blot analysis using anti-IFITM2 and anti-IFITM3 antibodies (**j**) SDS-PAGE and western blot analysis of whole cell lysates derived from mPB $CD34^+$ HSPCs pre-stimulated for 48 h. Immunoblotting was performed with anti-IFITM2 and anti-IFITM3 antibodies.

Supplementary Figure 7. **Caraphenol A treatment increases LV escape from endosome in HeLa does not alter early endosome number.** HeLa cells were plated and treated for 4 h with DMSO (0.06%), resveratrol (30μM) or caraphenol A (30μM) before addition of LV for 30 min or 2

h. Cells were then fixed, permeabilized and immunostained using α-Gag p24 and anti-early endosomal antigen 1 (EEA1) antibodies. Cells were analyzed using Imaris InCell software, identifying the number of (**a**) Gag p24 staining vesicles or (**b**) $EEA1^+$ endosomes. At least 50 cells were imaged per condition and plotted as dot plots (DMSO blue circles, resveratrol orange triangles, caraphenol A green circles, mean ± s.d), ****P<0.0001 by Kruskal-Wallis test with Dunn's multiple comparison correction. (**c**) mPB CD34+ HSPC were thawed, pre-stimulated for 48 h then treated for 4 h with either DMSO (0.06%) or caraphenol A (30μM) before addition of LV for 30 min or 2 hr. Cells were fixed, stained and analyzed as above for HeLa cells. (**d**) HeLa cells were pre-incubated for 2 h with LysoSensor pH sensitive dextran before addition of DMSO (0.06%, blue lines), resveratrol (30μM, orange lines), or caraphenol A (30μM, green lines). Cells were analyzed by flow cytometry for emission at 525nm (low pH) and 405nm (high pH) at indicated timepoints. Data is presented in linear plots (mean ± s.d) of the 525nm/405nm ratio normalized to DMSO (n=2 experiments, 4 cultures/point).

Supplemental References:

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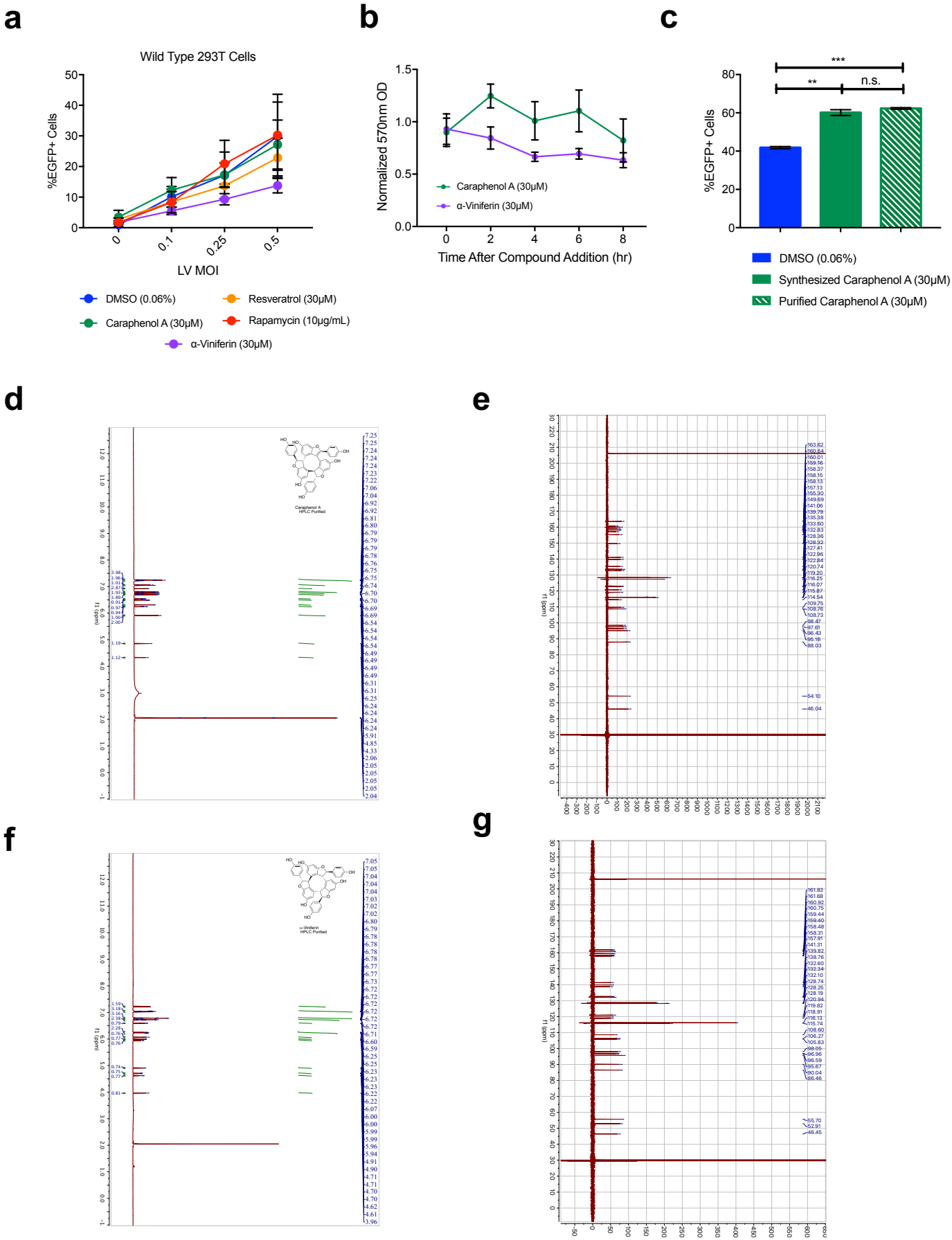
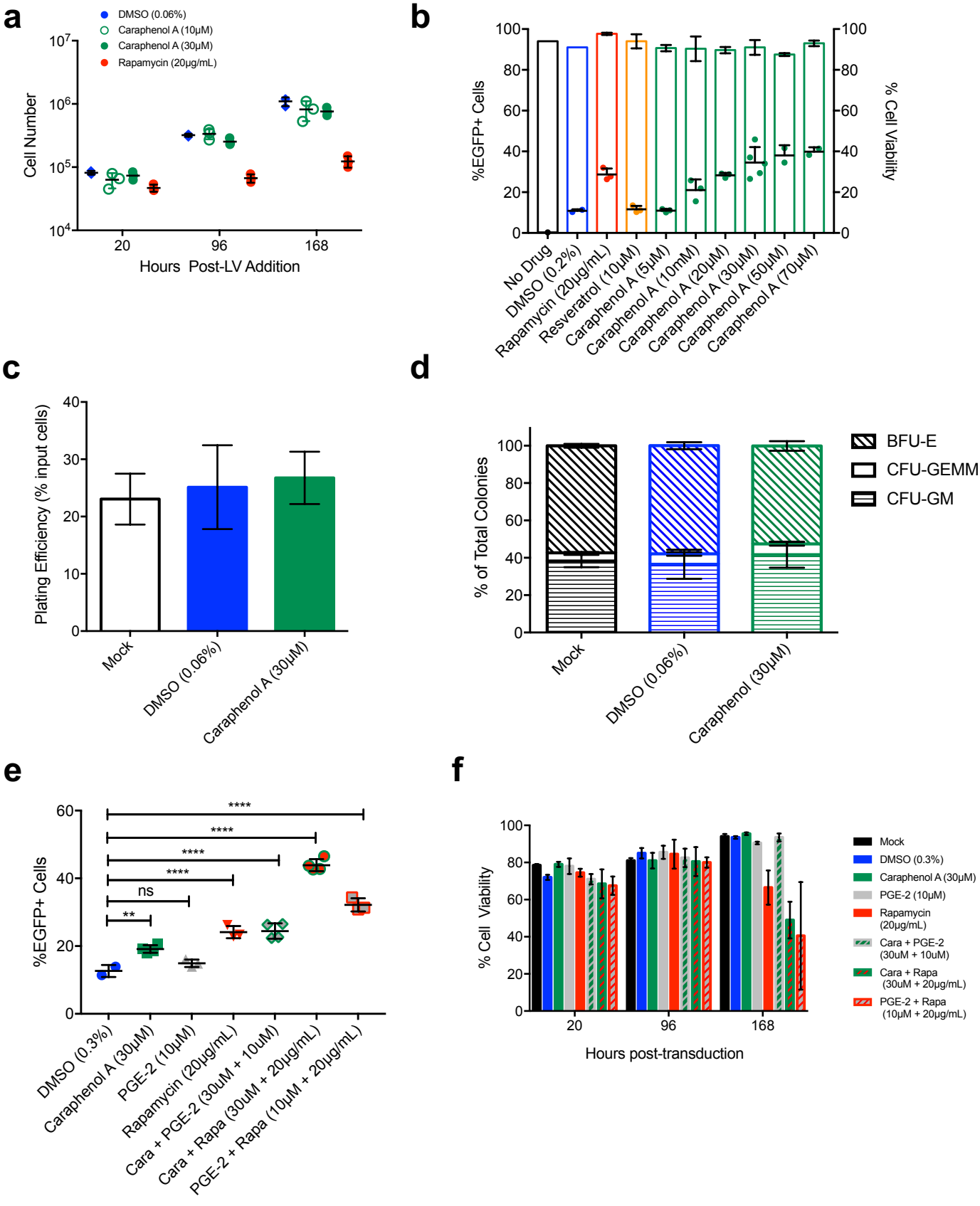


Supplemental Figure 1.

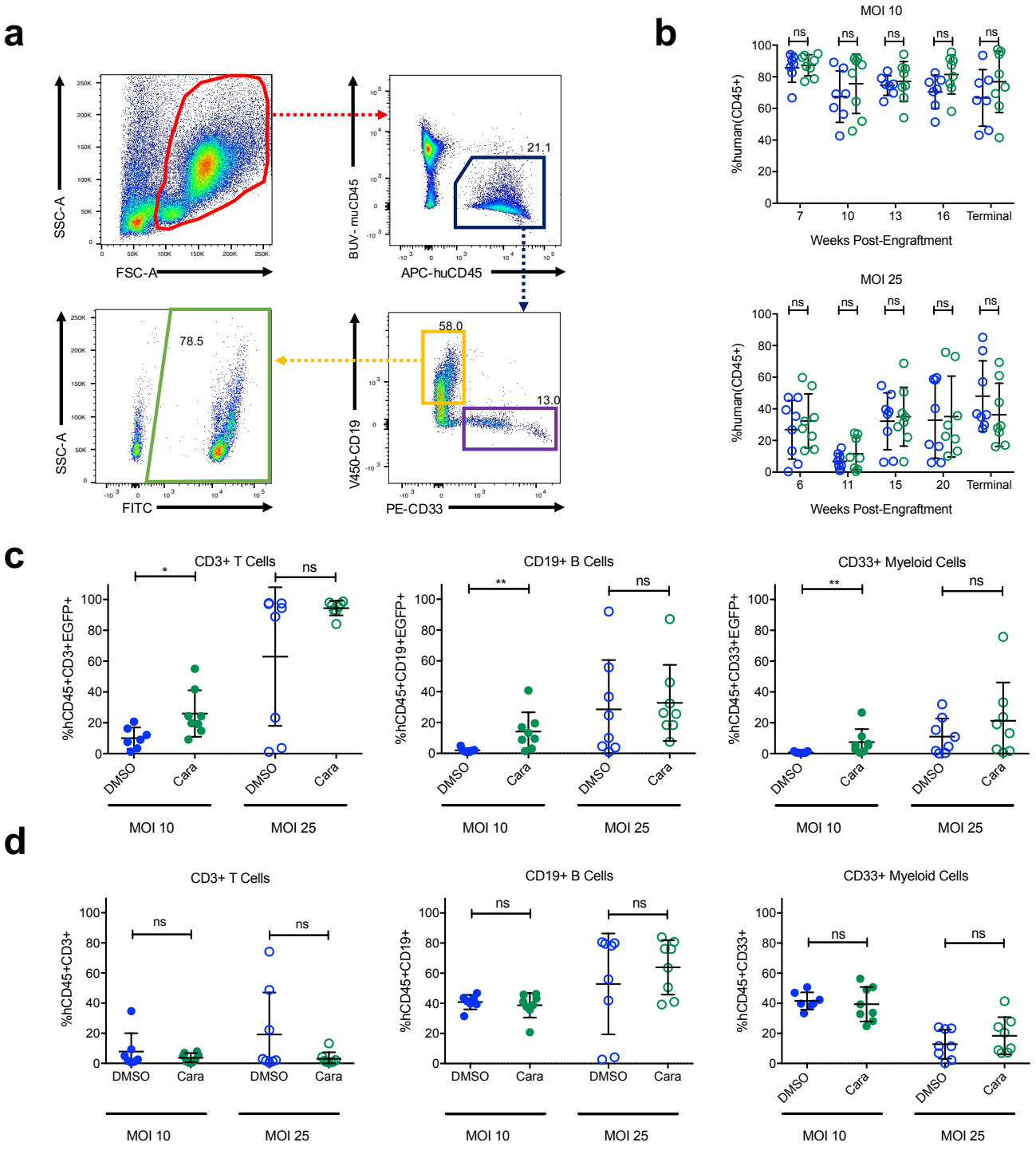
Tabulated Spectral Data: Caraphenol A		
^{1}H NMR in acetone-d_6		
Commercial (HPLC)	**Synthetic**	**Natural**
7.27–7.21 (m, 4H)	7.25 (d, J = 8.7 Hz, 2H)	7.26 (d, J = 8.7 Hz, 2H)
	7.23 (d, J = 8.4 Hz, 2H)	7.24 (d, J = 8.6 Hz, 2H)
7.07–7.03 (m, 2H)	7.05 (d, J = 8.6 Hz, 2H)	7.05 (d, J = 8.6 Hz, 2H)
6.92 (d, J = 2.0 Hz, 1H)	6.92 (d, J = 1.9 Hz, 1H)	6.94 (d, J = 1.8 Hz, 1H)
6.82–6.77 (m, 3H)	6.79 (d, J = 1.9 Hz, 1H)	6.81 (d, J = 1.8 Hz, 1H)
	6.79 (d, J = 8.6 Hz, 2H)	6.80 (d, J = 8.7 Hz, 2H)
6.77–6.73 (m, 2H)	6.75 (d, J = 8.8 Hz, 2H)	6.75 (d, J = 8.6 Hz, 2H)
6.71–6.68 (m, 2H)	6.69 (d, J = 8.6 Hz, 2H)	6.71 (d, J = 8.6 Hz, 2H)
6.54 (dd, J = 1.9, 1.1 Hz, 1H)	6.53 (d, J = 2.0 Hz, 1H)	6.54 (d, J = 1.8 Hz, 1H)
6.49 (dd, J = 2.2, 0.5 Hz, 1H)	6.49 (d, J = 2.0 Hz, 1H)	6.52 (d, J = 2.1 Hz, 1H)
6.31 (d, J = 2.2. Hz, 1H)	6.31 (d, J = 2.2 Hz, 1H)	6.32 (d, J = 2.1 Hz, 1H)
6.24 (dd, J = 2.1, 0.7 Hz, 1H)	6.24 (d, J = 2.0 Hz, 1H)	6.25 (d, J = 1.8 Hz, 1H)
5.91 (s, 2H)	5.91 (s, 2H)	5.92 (s, 1H)
		5.91 (s, 1H)
4.85 (s, 1H)	4.85 (s, 1H)	4.87 (s, 1H)
4.33 (s, 1H)	4.33 (s, 1H)	4.31 (s, 1H)
^{13}C NMR in acetone-d_6		
46.04	46.04	45.71
54.10	54.04	54.06
88.03	88.02	87.92
95.16	95.18	95.17
96.43	96.41	96.36
97.61	97.58	97.64
98.47	98.45	98.32
108.73	108.70	108.71
108.76	108.72	108.75
109.75	109.74	109.65
114.54	114.57	114.50
115.87	115.83	115.85
116.07	116.03	116.01
116.25	116.18	116.18
119.20	119.19	118.93
120.74	120.73	120.56
122.84	122.95	122.71
122.96	122.96	122.79
127.41	127.45	127.37
128.32	128.31	128.15
128.36	128.34	128.34
132.83	132.84	132.49
133.60	133.71	133.51
135.38	135.35	135.32
139.78	139.80	139.67
141.06	141.09	140.95
149.69	149.62	149.27
155.30	155.30	155.22
157.13	157.08	157.20
158.13	157.97	158.02
158.15	158.07	158.21
158.37	158.17	158.29
159.16	159.10	159.17
160.01	159.99	159.79
160.64	160.56	160.72
163.62	163.63	163.49
Natural and Synthetic data were obtained from Table S1 of Snyder's Caraphenol A Synthesis		

Tabulated Spectral Data: a-Viniferin	
^{1}H NMR in acetone-d_6	
Commercial (HPLC)	**Natural**
7.24–7.19 (m, 2H)	7.22 (d, J = 8.5 Hz, 2H)
7.06–6.99 (m, 4H)	7.08 (d, J = 8.5 Hz, 2H)
	7.03 (d, J = 8.5 Hz, 2H)
6.81–6.75 (m, 4H)	6.79 (d, J = 8.5 Hz, 2H)
	6.77 (d, J = 8.5 Hz, 2H)
6.74–6.68 (m, 3H)	6.72 (d, J = 8.5 Hz, 2H)
	6.72 (d, J = 1.8 Hz, 1H)
6.59 (d, J = 2.1 Hz, 1H)	6.59 (d, J = 1.8 Hz, 1H)
6.26–6.21 (m, 3H)	6.25 (d, J = 1.8 Hz, 1H)
	6.22 (d, J = 1.8 Hz, 1H)
	6.22 (d, J = 1.8 Hz, 1H)
6.07 (s, 1H)	6.07 (bs, 1H)
5.99 (dd, J = 2.2, 0.6 Hz, 1H)	5.99 (d, J = 1.8 Hz, 1H)
5.95 (d, J = 9.9 Hz, 1H)	5.95 (d, J = 9.7 Hz, 1H)
4.91 (d, J = 6.3 Hz, 1H)	4.90 (d, *J* = 6.4 Hz, 1H)
4.71 (dd, J = 9.9, 0.9 Hz, 1H)	4.71 (d, *J* = 9.7 Hz, 1H)
4.62 (d, J = 6.2 Hz, 1H)	4.61 (d, *J* = 6.4 Hz, 1H)
3.96 (s, 1H)	3.97 (bs, 1H)
^{13}C NMR in acetone-d_6	
46.45	46.4
52.91	52.8
55.70	55.6
86.46	86.4
90.04	90.0
95.67	95.6
96.59	96.6
96.96	96.9
98.05	98.0
105.83	105.8
106.27	106.2
108.60	108.5
115.74	115.7
116.13	116.1
	116.1
118.91	118.8
119.82	119.7
120.94	120.9
128.19	128.1
128.25	128.2
128.74	128.6
132.10	132.0
132.34	132.3
132.60	132.5
138.76	138.7
139.82	139.7
141.31	141.2
157.91	157.8
158.31	158.2
158.48	158.3
159.40	159.3
159.44	159.3
160.75	160.6
160.92	160.8
161.68	161.6
161.83	161.7
Natural data taken from Kitanaka, S. et al. Chem. Pharm. Bull. 1990, 38, 432	

Supplemental Table 1.



Supplemental Figure 2.



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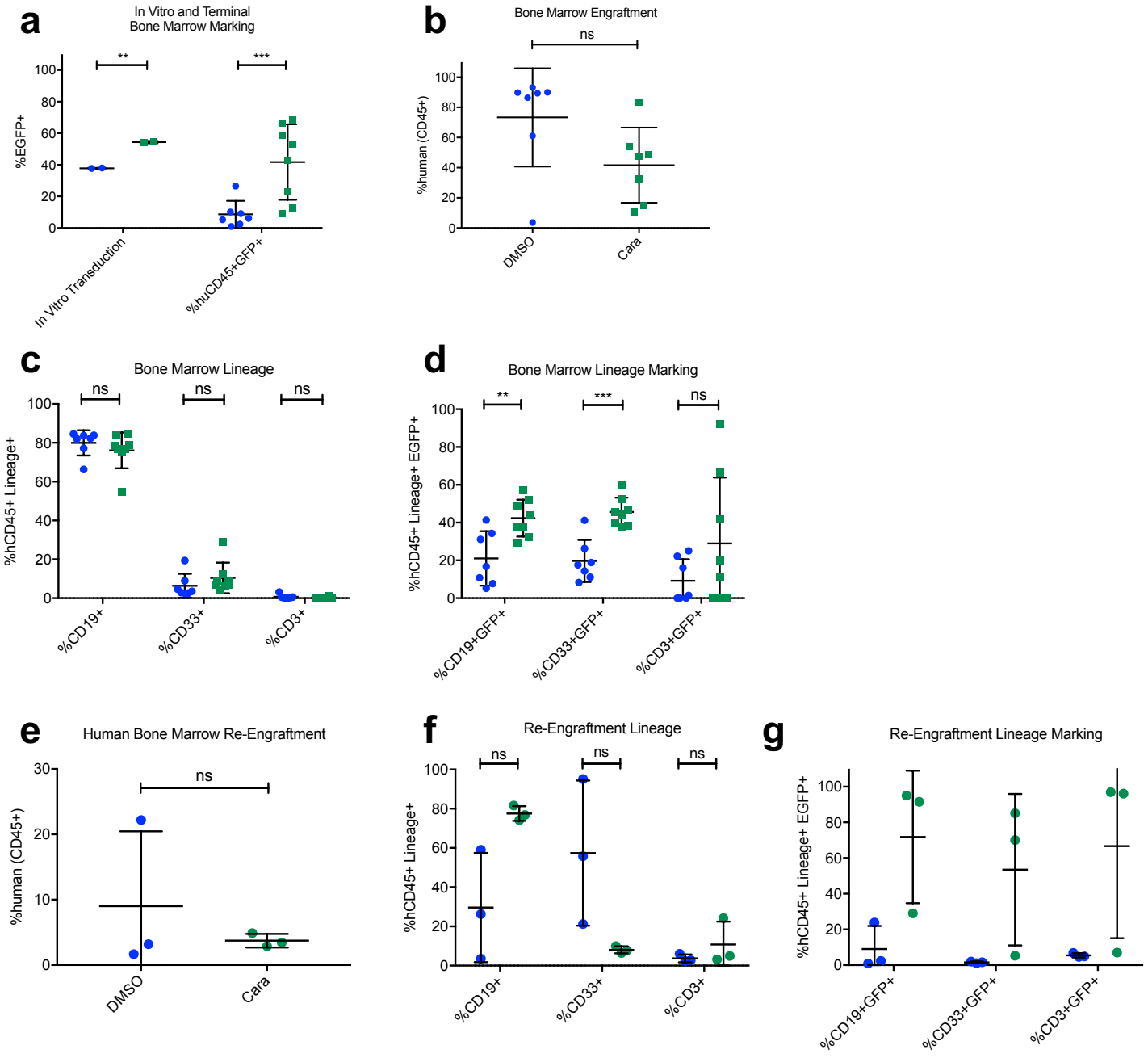
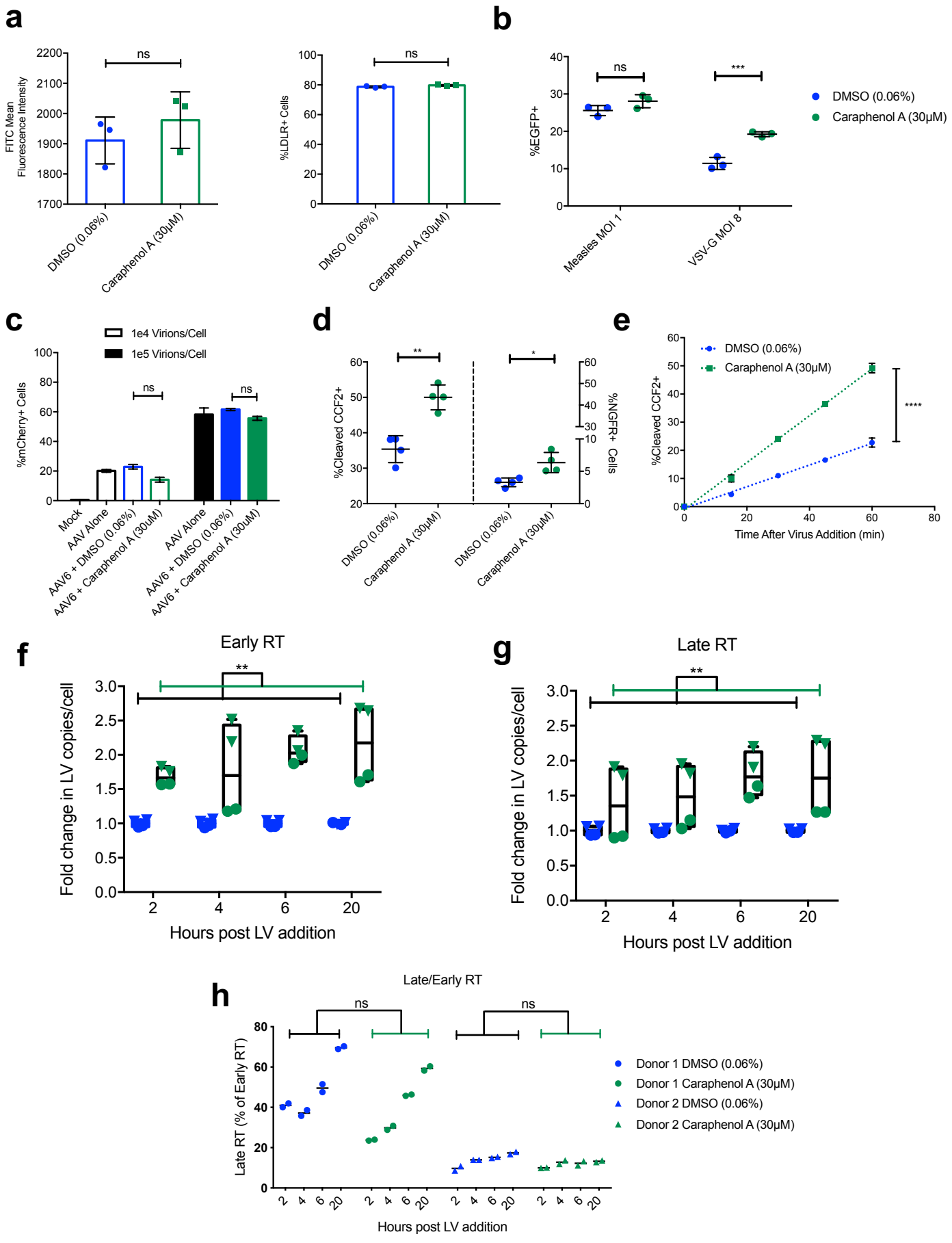
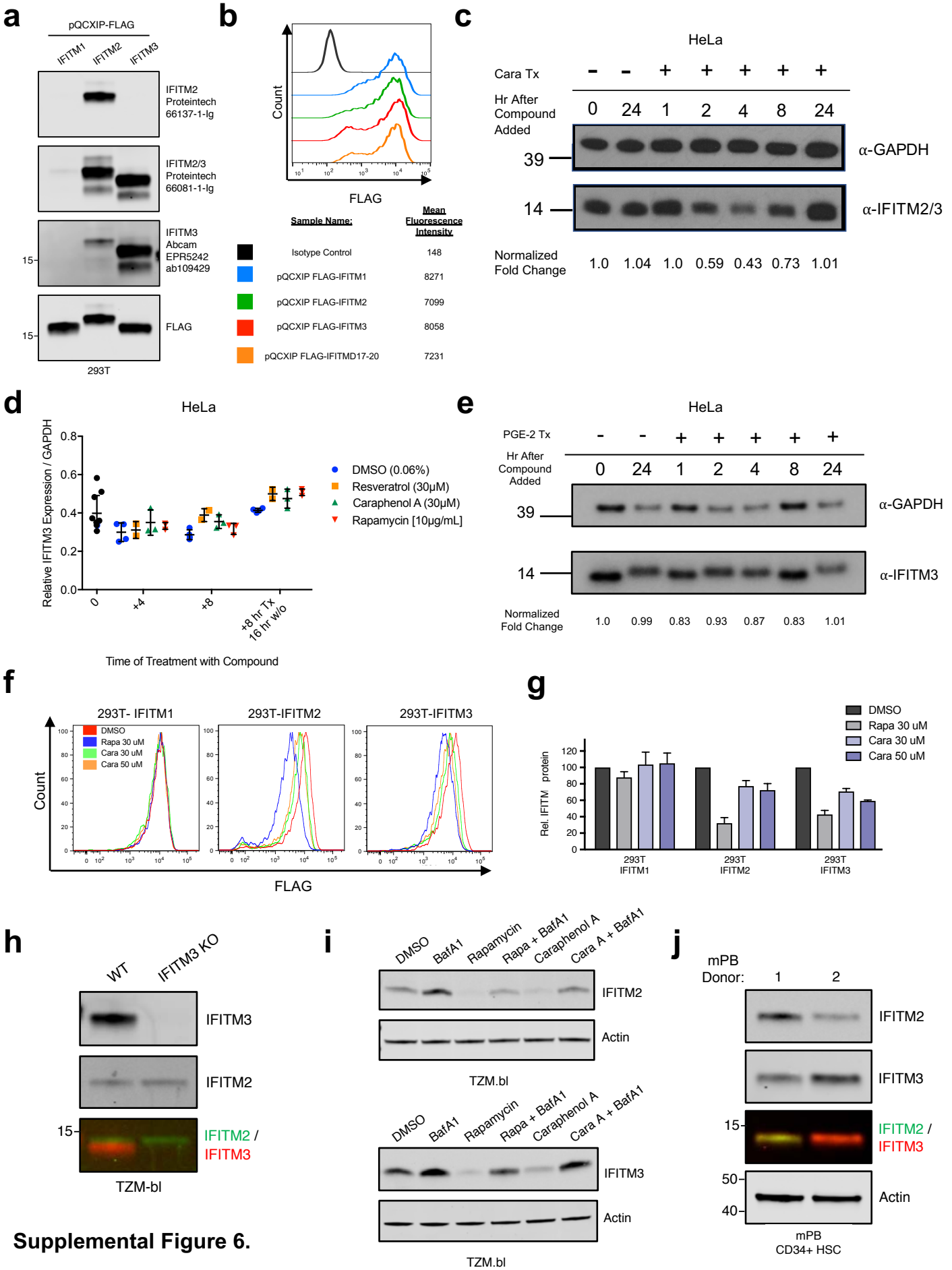


Supplemental Figure 4.



Supplemental Figure 5.



Supplemental Figure 6.