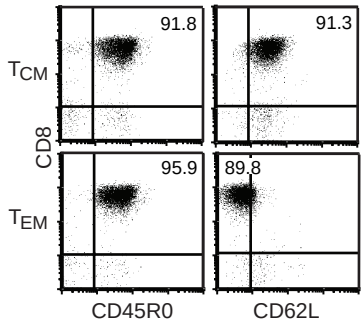
Figure S1. Purifity of sorted $CD8^+$ T_{CM} and T_{EM} phenotype
Washed PBMC were first autoMACS™ depleted using anti-CD45RA, anti-CD4, and anti-CD14 beads (Miltenyi Biotec) per the manufacturer's protocol. The negative fraction was then labeled with PE-conjugated anti-CD4RO, FITC-conjugated anti-CD62L, and APC-conjugated anti-CD8 and underwent FACS for the $CD8^+CD62L^+CD45RO^+$ T_{CM} and $CD8^+CD62L^-CD45RO^+$ T_{EM}. Histogram quadrants in each case are based on staining with fluorochrome-conjugated isotype controls.

Figure S2. Generation of vAPCs that have been genetically modified to express CMVpp65 antigen
(A) Plasmid construct used to drive CMVpp65 expression in vAPC cells contains a fusion of the hygromycin-resistance and pp65 genes (Hypp65) driven by the human EF1 promoter (hEF1p). (B) Successful pp65 expression in U293T cells that have been electrotransferred with this plasmid is depicted by immunohistochemistry. Briefly, U293T cells were transfected with pp65plasmid DNA-lipofectamine complex (lipofectamine 2000, Invitrogen). 24 h after transfection, cells were fixed with 5% acetone in EtOH and stained with anti-pp65 mouse mAb (Leica Biosystems, Bannockburn, IL) followed by biotinylated horse anti-mouse secondary Ab (Leica Biosystems) and avidin-biotin peroxidase development (Vectastain ABC kit, Vector Laboratories, Inc., Burlingame, CA). (C) Efficiency of electrotransfer is determined with co-transferred GFP expressing plasmid construct in viable PBMC population. CD3 staining vs. GFP is depicted in donor PBMC (left) and one day after electroporation (right, vAPC).

Figure S3. Engraftment of EBV-specific $T_{CM/E}$ is greater than that of $T_{EM/E}$
(A) Schematic of EBV-antigen specific $T_{CM/E}$ and $T_{EM/E}$ derivation. Purified T_{CM}, T_{EM}, and pp65-expressing vAPC were generated from the same healthy EBV-seropositive donor's PBMC. The T_{CM} and T_{EM} were then each co-incubated with lethally irradiated autologous LCL at a responder:stimulator ratio of 4:1. The cultures were stimulated three times in this manner every 7 days with 5U/mL IL-2 supplemented every other day. (B) After three stimulations, cytotoxic activity of these $T_{CM/E}$ and $T_{EM/E}$ against autologous or allogeneic LCL as targets was determined in a 4hr chromium release assay. Mean percent ^{51}Cr release ± S.D. of triplicate wells is depicted. (C) For the engraftment studies, 4×10^6 EBV-specific $T_{CM/E}$ and $T_{EM/E}$ were administered i.p. on day 0. Antigen stimulation in the form of irradiated auto-LCL was provided along with irradiated IL-15 secreting NS0 cells on days 19 and 32. Peripheral blood was harvested by retro-orbital bleeding and mean percent human T cells ($CD45^+$ $CD8^+$) ± S.E. was determined by flow cytometry. *, $p < 0.05$ when comparing $T_{CM/E}$ values to $T_{EM/E}$ values using ANOVA.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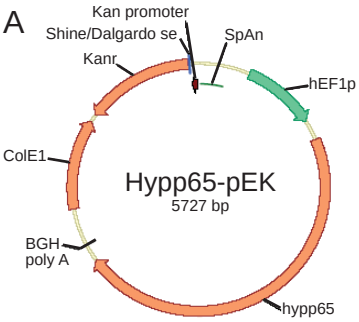
91.3
95.9
89.8
TCM
TEM
CD8
CD45R0
CD62L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Kan promoter
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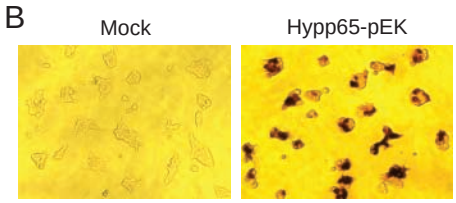
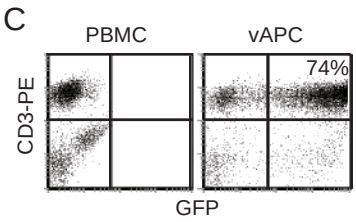
SpAn
Kanr
hEF1p
ColE1
Hypp65-pEK
5727 bp
BGH
poly A
hypp65


B
Mock
Hypp65-pEK


C
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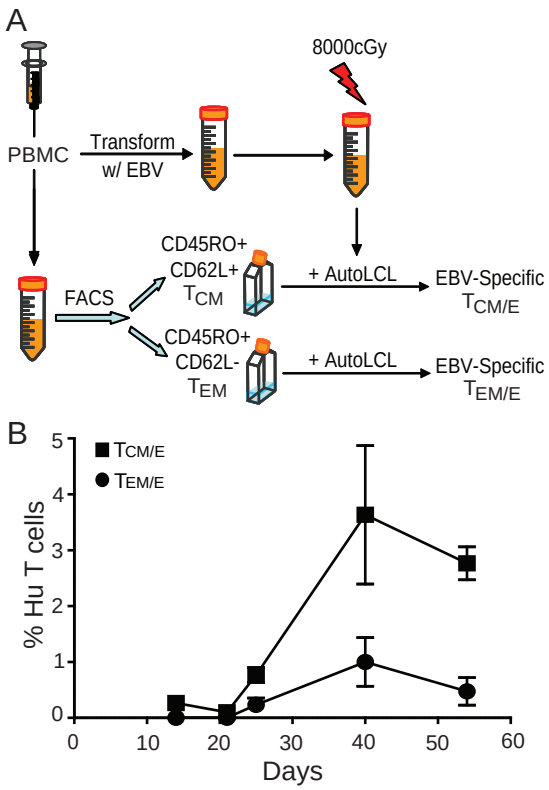
vAPC
74%
CD3-PE
GFP


A
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PBMC
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w/ EBV
CD45RO+
CD62L+
TCM
+ AutoLCL
EBV-Specific
TCM/E
FACS
CD45RO+
CD62L-
TEM
+ AutoLCL
EBV-Specific
TEM/E
B
TCM/E
TEM/E
% Hu T cells
0
1
2
3
4
5
0
10
20
30
40
50
60
Days