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The V86M mutation in HIV-1 Capsid Confers Resistance to TRIM5α by Abrogation of Cyclophilin A-Dependent Restriction and Enhancement of Viral Nuclear Import.

Supplementary data

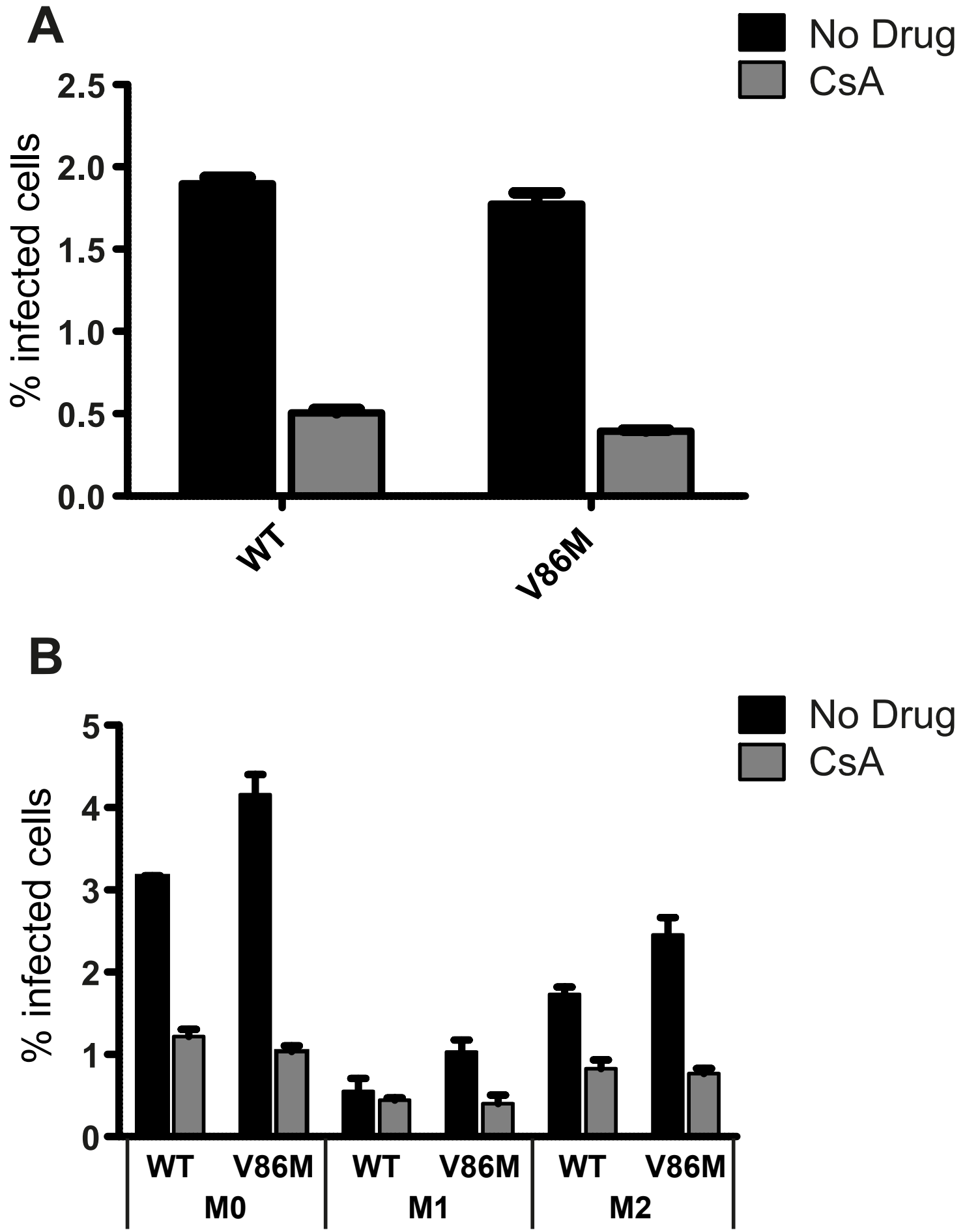
Figure S1. CA-V86M HIV-1 is inhibited by CsA in human lymphocytes and macrophages. (**A**) Activated human $CD4^+$ lymphocytes were challenged with WT or V86M HIV-$1_{NL\text{-}GFP}$ at a multiplicity of infection of ~0.1 in the absence or presence of 2 μM CsA. WT and V86M viral preparations were adjusted by reverse transcriptase assay. The percentage of infected (GFP-positive) cells was determined two days later by flow cytometry. Shown are average values from three independent infections with standard deviations. (**B**) THP-1 cells differentiated and polarized into either macrophages (M0), pro-inflammatory macrophages (M1) or anti-inflammatory macrophages (M2) were challenged with WT or V86M HIV-$1_{NL\text{-}GFP}$ exactly like above but at an M.O.I of ~0.02.

Figure S2. Western blotting analysis of CypA knockdown. TE671 cells (top) and Sup-T1 cells (bottom) expressing the indicated TRIM5α_{hu} mutants and controls were stably transduced with retroviral vectors expressing shRNAs targeting either CypA or the non-relevant control Luciferase. Untransduced cells were eliminated; then, whole cell lysates were prepared from a similar numbers of cells and processed for western blotting using antibodies against CypA or X-actin as a loading control.

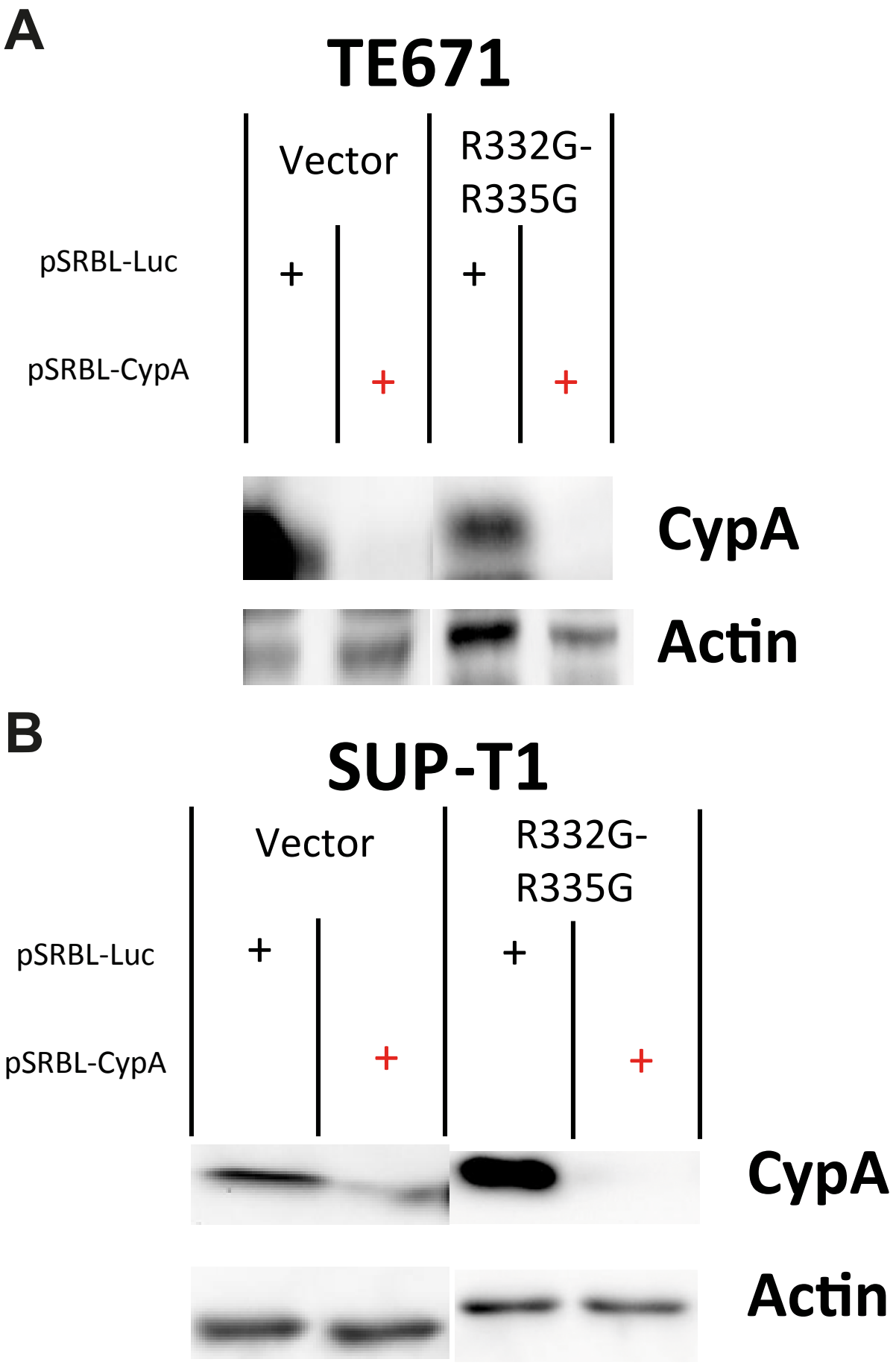
Figure S3. Restriction of WT and CA-V86M HIV-1 by a panel of TRIM5α_{hu} mutants. (**A**) TE671 cells transduced with the indicated TRIM5α cDNAs were challenged with multiple doses of WT or CA-V86M HIV-$1_{NL\text{-}GFP}$ as described in Figure

1. Cells were analyzed by FACS 2 days later. Control permissive cells transduced with the "empty" vector and infected with WT HIV-$1_{NL\text{-}GFP}$ are included. (**B**) TE671 cells expressing the indicated TRIM5α_{hu} mutants and controls were challenged with WT or V86M HIV-$1_{NL\text{-}GFP}$ in the presence of increasing concentrations of CsA. Virus doses were adjusted for each virus-cell combination so that approximately 1% of the cells were infected in the absence of CsA. The percentage of GFP-positive cells was determined by FACS 2 days later and results are shown as –fold increases relative to the no-drug controls.

S1

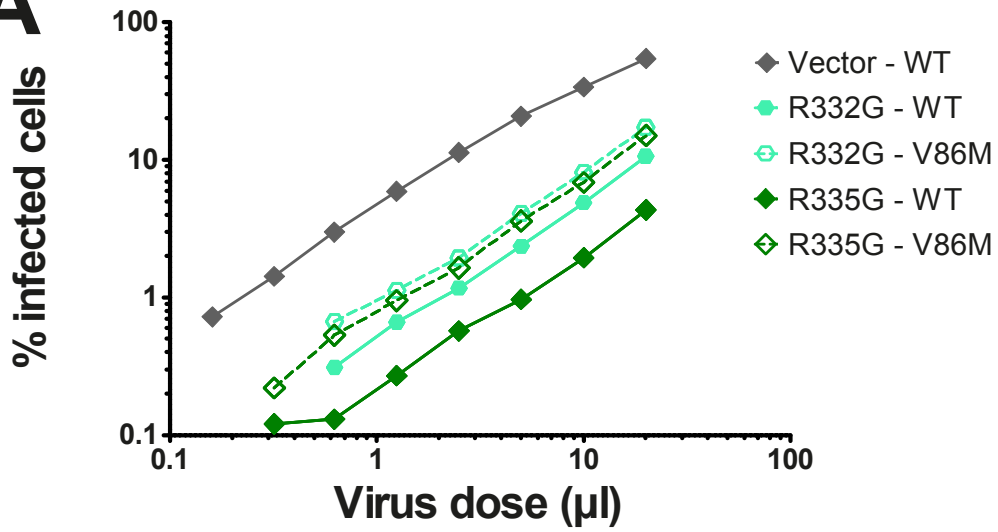

A
No Drug
CsA
% infected cells
2.5
2.0
1.5
1.0
0.5
0.0
WT
V86M
B
No Drug
CsA
% infected cells
5
4
3
2
1
0
WT
V86M
WT
V86M
WT
V86M
M0
M1
M2

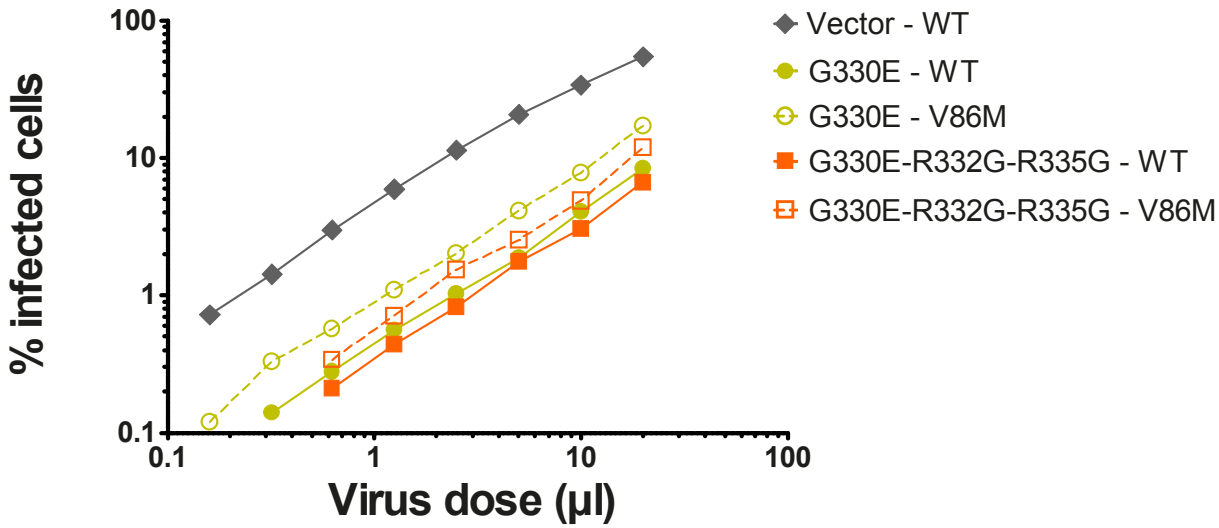
S2


A
TE671
Vector
R332G-R335G
pSRBL-Luc
pSRBL-CypA
+
+
+
+
CypA
Actin
B
SUP-T1
Vector
R332G-R335G
pSRBL-Luc
pSRBL-CypA
+
+
+
+
CypA
Actin

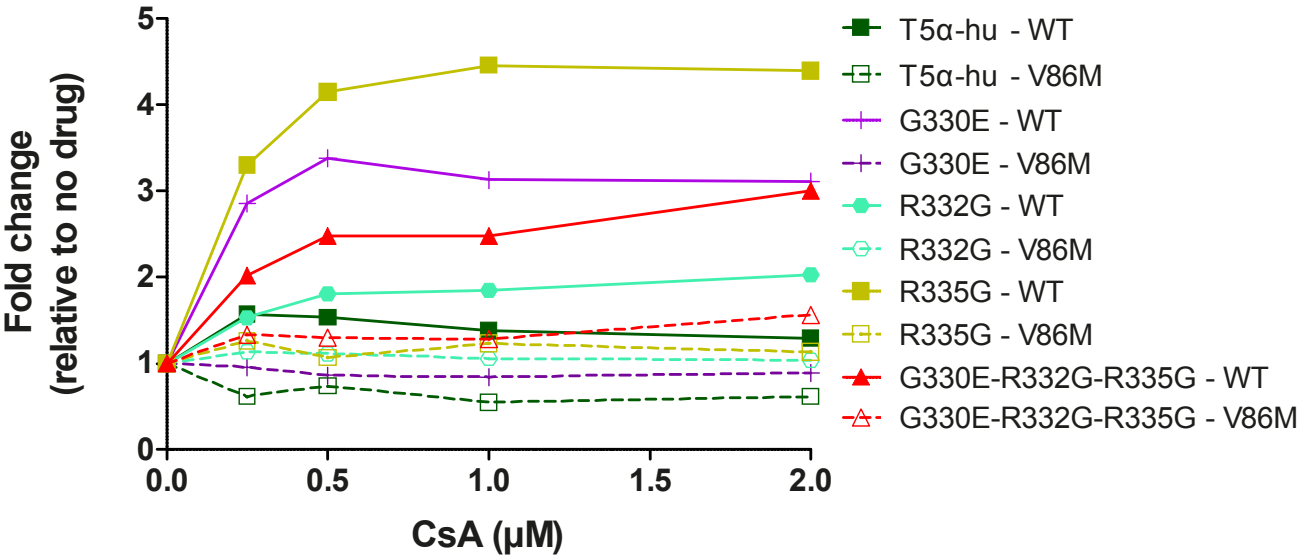
S3

A


% infected cells
100
10
1
0.1
0.1
1
10
100
Virus dose (µl)
Vector - WT
R332G - WT
R332G - V86M
R335G - WT
R335G - V86M


% infected cells
100
10
1
0.1
0.1
1
10
100
Virus dose (µl)
Vector - WT
G330E - WT
G330E - V86M
G330E-R332G-R335G - WT
G330E-R332G-R335G - V86M

B


Fold change
(relative to no drug)
5
4
3
2
1
0
0.0
0.5
1.0
1.5
2.0
CsA (µM)
T5α-hu - WT
T5α-hu - V86M
G330E - WT
G330E - V86M
R332G - WT
R332G - V86M
R335G - WT
R335G - V86M
G330E-R332G-R335G - WT
G330E-R332G-R335G - V86M