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Supplementary Materials for

HIV-1 Vpr Induces Adipose Dysfunction in Vivo Through Reciprocal Effects on PPAR/GR Co-Regulation

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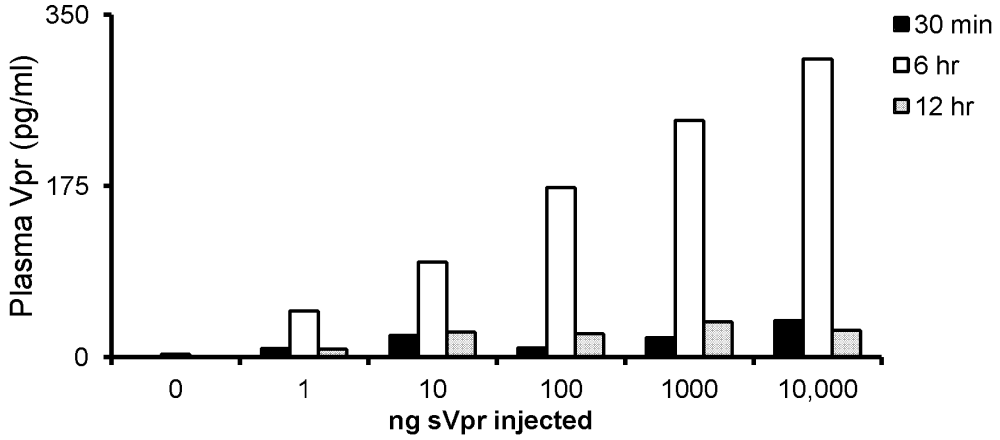
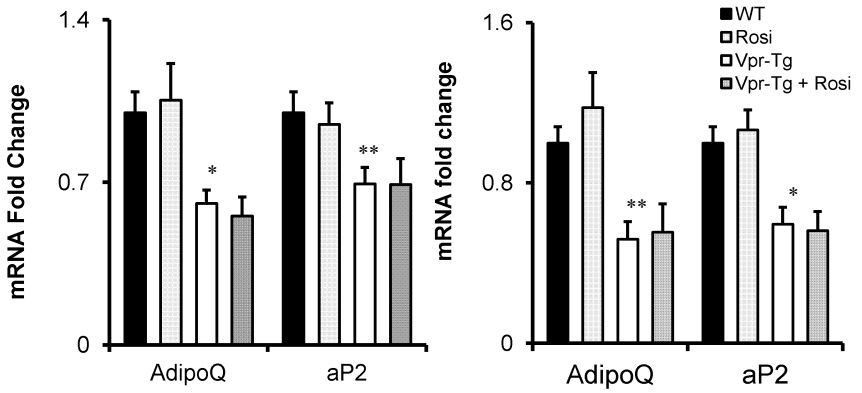
The PDF file includes:

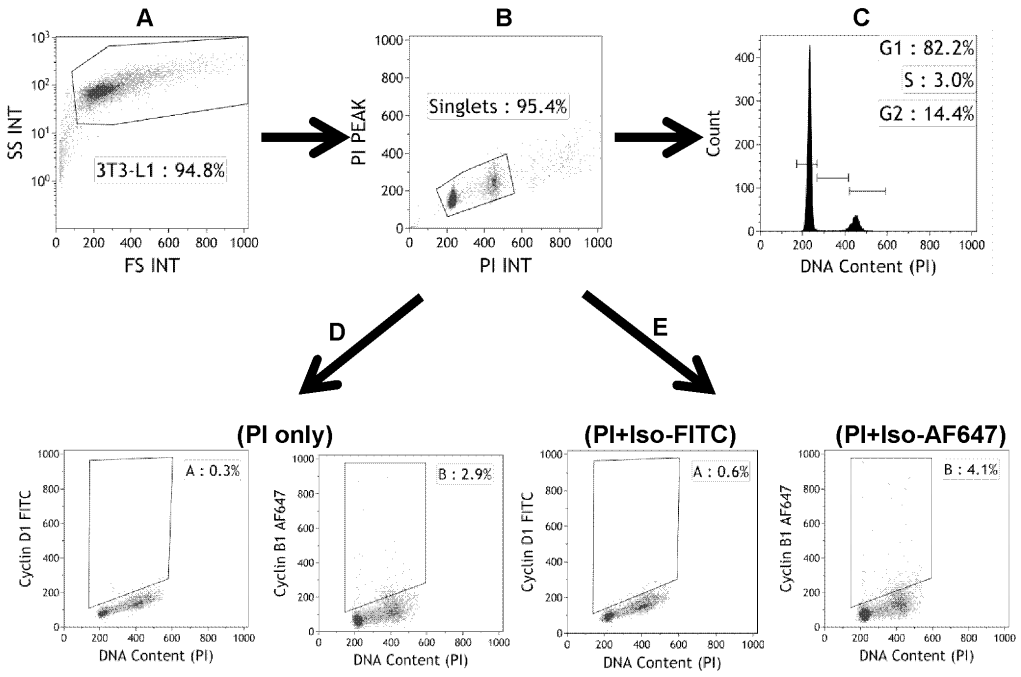
Fig. S1. Plasma Vpr levels in mice injected with sVpr.
Fig. S2. Expression of adiponectin and aP2 in PGF and IF of Vpr-Tg.
Fig. S3. Gating strategy for flow cytometry.
Fig. S4. Vpr does not block differentiation genes in 3T3-L1 72 hours after differentiation induction.
Fig. S5. Hyperglycemia and hypertriglyceridemia in Vpr-Tg.
Fig. S6. Paracrine effect of Vpr.
Table S1. Vpr in human liver and adipose tissue.
Table S2. Palmitate flux in a second line of Vpr-Tg.
Table S3. Twenty-four–hour calorimetry.
Table S4. Mouse weight and food intake in Vpr-Tg and sVpr-treated mice.
Table S5. Primer sequences for ChIP qPCR.
Reference (*49*)



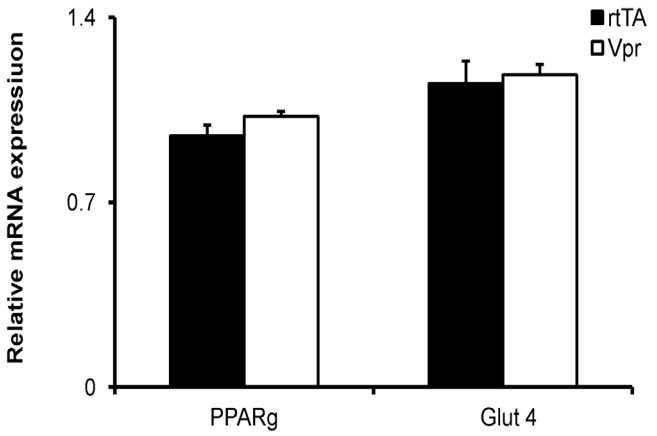
Supplementary Figure S1: Plasma Vpr levels in mice injected with sVpr.



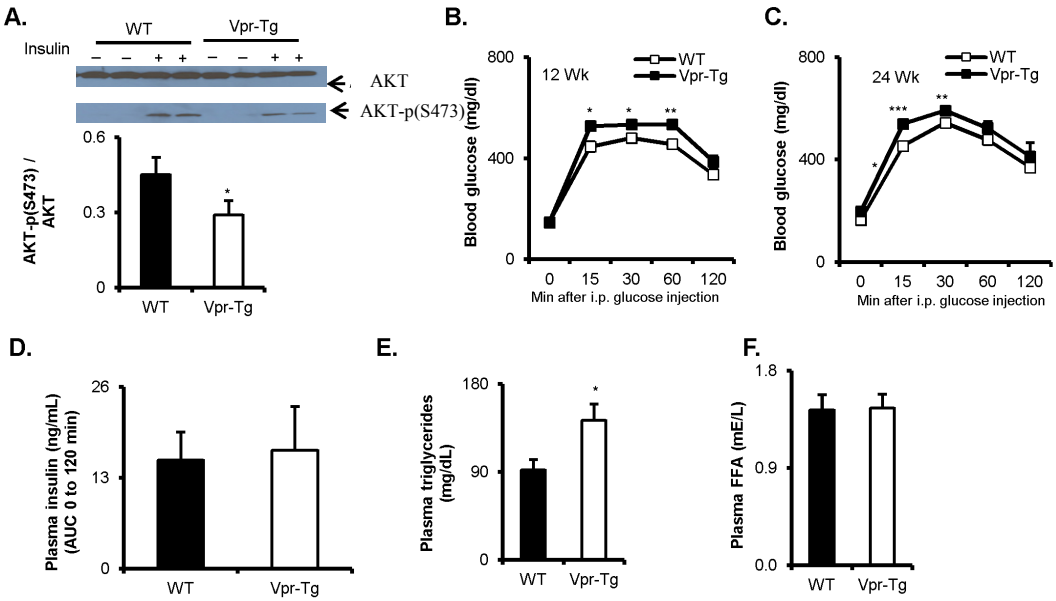
Supplementary Figure S2: Expression of adiponectin and aP2 in PGF and IF of Vpr–Tg. A. Reduced *AdipoQ (P = 0.02) and ap2 (P = 0.009)* mRNA expression in inguinal fat (IF) of Vpr-Tg compared to WT mice ; rosiglitazone treatment had no effect. N=8 per group. **B.** Reduced A*dipoQ (P = 0.01) and ap2 (P = 0.03)* mRNA expression in perigonadal fat (PGF) of Vpr-Tg compared to WT mice ; rosiglitazone treatment had no effect. N=8 per group. $^{*}P < 0.05$, $^{**}P < 0.01$



Supplementary Figure S3. Gating strategy for flow cytometry. Live cells were gated from light scatter dotplot (**A**), and single cells examined by PI doublet discrimination (**B**). Single cells were then examined by one-parameter PI histogram for overall cell cycle distribution (**C**), or cell cycle in conjunction with either cyclin D1-FITC or cyclin B1-AF647. Positivity of cyclins in conjunction with PI was determined based on cells stained with PI only (**D**) or PI+Isotype-FITC or PI+Isotype-AF647 (**E**) controls.

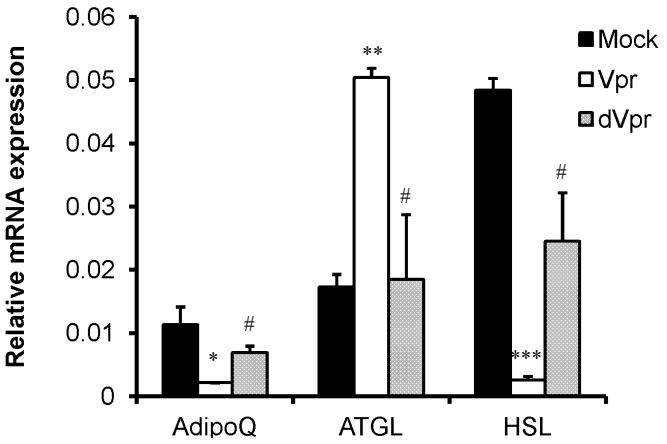


Supplementary Figure S4: Vpr does not block differentiation genes in 3T3-L1 72 hours after differentiation induction. PPARγ and Glut4 expression on day 8 in 3T3-L1 adipocytes 72h after doxycycline addition.



Supplementary Figure S5: Hyperglycemia and hypertriglyceridemia in Vpr-Tg. **A.** Reduced insulin-stimulated phospho-Ser473 Akt levels, as a fraction of total Akt protein level, in Vpr-Tg (N=5) compared to WT (N=5) mice (p=0.05). **B and C.** Intraperitoneal glucose tolerance tests show post-challenge hyperglycemia in Vpr-Tg compared to WT mice at 14 weeks of age (N=5 for WT mice and N=4 for Vpr-Tg mice) (p=0.02, 0.03 and 0.01 at 15, 30, 60 min respectively) (b), and at 24 weeks of age (N=7 for WT mice and N=5 for Vpr-Tg mice) (p=0.03, 0.001 and 0.01 at 0, 15, 30 min respectively) . **D.** Plasma insulin levels during GTT in WT (N=7) and Vpr-Tg (N=5) mice at 24 weeks of age. **E.** Elevated fasting plasma triglyceride levels in Vpr-Tg (N=9) compared to WT (N=11) mice (p=0.02). **F.** Plasma FFA levels in Vpr-Tg (N=9) compared to WT (N=11) mice. Values are mean ± SE.

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.



Supplementary Figure S6. Paracrine effect of Vpr. Memory CD4+ T cells (isolated from human PBMC's) were either uninfected or infected with wild-type HIV NL4.3 strain or Vpr-deficient NL4.3 for 24h in IL2-containing medium. After the infection period, $2x10^5$ memory CD4+ T cells were washed and placed in transwells with $2x10^5$ primary human subcutaneous preadipocytes (Zen-Bio, RTP, NC) in the lower wells, and cocultured in preadipocyte maintenance medium (Zen-Bio PM-1) containing IL2 (10 ng/ml). After 6 days coculture, the preadipocytes were hormonally induced for terminal differentiation by switching to preadipocyte differentiation medium (Zen-Bio DM-2) with IL2. After 3 days coculture in differentiation medium, the transwells were removed, the differentiating adipocytes harvested and their mRNA extracted for qRT-PCR measurements of *AdipoQ* (adiponectin, PPARγ target), *Atgl* (GR target), and *Hsl* (GR target). Compared to exposure to activated, uninfected T cells ("Mock"), exposure to wt-HIV-infected T cells ("Vpr") decreased expression of *AdipoQ* (p=0.04) and *Hsl* (p=0.0009) but increased expression of *Atgl* (p=0.002), consistent with the findings in inguinal (subcutaneous) fat depots of WT compared to Vpr-Tg mice (**Figs. 3C, 3F**). Exposure to Vpr-deficient-HIV-infected T cells ("dVpr") restored the levels of expression of these three genes to those of the basal ("Mock") condition of exposure to activated T cells alone (p=0.02, 0.05, 0.04 for *AdipoQ*, *Hsl* and *Atgl* respectively). HIV replication was similar in the wt-HIV-infected and Vpr-deficient-HIV-infected T cells, as assessed by p24 production. Data are representative of 3 experiments. Values are mean ± SD.

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

$^{\#}P < 0.05$

* Comparison between Mock (activated, uninfected T cells) condition and wt-HIV-infected condition

Comparison between WT-HIV-infected condition and Vpr-deficient-HIV-infected condition

Supplementary Table S1. Vpr in humn liver and adipose tissue.

Patient	HIV Status	Tissue	Vpr sequence detected
80y, M	Non-HIV	Spleen	None
84y, M	Non-HIV	L. Node	None
64y, M	Non-HIV	Fat	None
		Liver	None
39y, F	HIV	Spleen	None
		L. Node	None
		Fat	MEQAPEDQGPQ
		Liver	MEQAPEDQGPQR
64y, M	HIV	Spleen	None
		L. Node	None
		Fat	MEQAPEDQGPQREPHNEWTL
		Liver	MEQAPEDQGPQRREPHNEWTLELLEE

Fresh, coded autopsy specimens were obtained from National Development and Research Institute (NY, NY) and analyzed in a blinded fashion. The tissues were homogenized in PBS / 0.1% SDS and the supernatants injected into a multi-dimensional micro-HPLC system, fractionated through a strong anion exchange column, and the eluting peaks passaged through a C18 reverse-phase column. Individual peaks were digested by trypsin and cyanogen bromide, reprocessed through a C4 reverse-phase column and recollected in micro-fractions. These samples were placed in a nanoflow electrospray interface to a dual quadrupole mass spectrometer, and peptide sequences obtained from the mass spectrometric analysis were fed into the SwissProt database for matches to known Vpr sequences.
(Full Vpr sequence: MEQAPEDQGPQREPHNEWTLELLEELKNEAVRHFPRIWLHGLGQHIYETYGDTWAGVEARIIRILQQLLF IHFRIGCRHSREGVTRQRRNASRS)

Supplementary Table S2. Palmitate flux in a second line of Vpr-Tg.

	WT (SE)	N	Vpr-Tg (SE)	N	P
Palmitate flux (µmoles/kg/hr)	3176 ± 291	3	2933 ± 51	3	0.49

Supplementary Table S3. Twenty-four-hour calorimetry.

	WT (SE)	**N**	**Vpr-Tg (SE)**	**N**	**P**
Mean RER (regular diet)	0.85 ± 0.006	10	0.84 ± 0.009	10	0.24
Mean RER (high (60%) fat diet)	0.80 ± 0.005	11	0.80 ± 0.005	12	0.9

Mean RER represents mean of measurements made every 10 min in each mouse over 24h.

Supplementary Table S4. Mouse weight and food intake in Vpr-Tg and sVpr-treated mice.

Groups (N)	**Avg. Mouse Weight ± SE**	**P**	**Avg. Food Intake in gms / week ± SE**	**P**
WT (8)	27.1 ± 0.599		37.4 ± 0.499	
Vpr-Tg (5)	26.9 ± 0.380	0.82	38.2 ± 0.342	0.25
Vehicle (6)	29.2 ± 0.30		42.0 ± 0.9	
sVpr (7)	30.5 ± 0.70	0.13	41.3 ± 0.7	0.53

Supplementary Table S5. Primer sequences for ChIP qPCR.

Gene Target	Forward Primer	Reverse Primer	Ref.
Adiponectin (PPRE)	5'TGTTGTTGACTCTCCAGGAC3'	5'TAGAGCTTCTGTCAAGCCAT3'	49
Ap2 (PPRE)	5'CAAGCCATGCGACAAAGGCA3'	5'TAGAAGTCGCTCAGGCCACA3'	48
Hsl (*Lipe*) (GRE)	5'GCCTGTTCAGCTGACAAGTG3'	5'TGGGTAAGTATGGCAGGAGTG3'	39
Adiponectin (off-target)	5'CTGTGGGGAAATCTGGCCTT3'	5'CAGAGTGAAGCAGCCACAGA3'	*
Ap2 (off-target)	5'CCTGCCTCACAGCAGAATGA3'	5'GGGAGCAGGTACTTCTCAGC3'	*
Hsl (*Lipe*) (off-target)	5'CCATGTGTCTGGCATTCCCT3'	5'GGCATCTGTAGGGCTGTTGT3'	*

DNA primers for gene target sites were designed based on locations provided by the referenced publications within the respective assemblies (mm8 for PPRE, mm9 for GRE). Off-target control primers for verification of specificity of the immunoprecipitation were designed to be within 2 kb from the binding sites chosen, without evidence for PPARγ or GR binding by referenced Chip-Seq data, and primer-blast (NCBI, Build 38.1) was used to avoid non-specific amplification products.