

Supplementary Figure 1:

Statistical analyses

Figure 3

We are interested in comparing log₁₀ RLU of pGL3-Luciferase activity (Figure 3D) and hMRC1 basic promoter activity (Figure 3E) between column 1 and columns 2, 3, 4, and 5.

Figure 3D

Comparisons of pGL3-Luciferase Activity - Figure 3D

2	3	4	5
0.663	0.895	0.646	0.935

p values are from a t test comparing column 1 to each group presented in the table above
RLU values were log₁₀ transformed

Figure 3E

Comparisons of hMRC1 Basic Promoter Activity - Figure 3E

2	3	4	5
0.874	0.862	0.332	0.64

p values are from a t test comparing column 1 to each group presented in the table above
RLU values were log₁₀ transformed

Figure 4

Figure 4E

In Figure 4E, we are interested in comparing firefly luciferase (measured in RLU) between Firefly(+)/Renilla(-) and Firefly(+)/Renilla(+) at 4 different concentrations (ug DNA) of PU.1. RLU values are log10 transformed.

Comparisons of Firefly Luciferase - Figure 4E

0	0.02	0.06	0.2
0.532	0.195	0.376	0.266

p values are from a t test comparing firefly luciferase (RLU) between firefly(+)/renilla(-) and firefly(+)/renilla(+) at various ug DNA of PU.1
RLU values were log10 transformed

Figure 4F

In Figure 4F, we are interested in comparing firefly luciferase (measured in RLU) in Firefly(+)/Renilla(+) between different concentrations (ug DNA) of PU.1. We compare 0 ug DNA to 3 other concentrations: 0.02, 0.06, and 0.2. RLU values are log10 transformed.

Comparisons of Firefly Luciferase - Figure 4F

0.02	0.06	0.2
0.086	0.545	0.131

p values are from a t test comparing firefly luciferase (RLU) in firefly(+)/renilla(+) between 0 ug PU.1 DNA and other concentrations in the table above
RLU values were log10 transformed

Figure 5

Figure 5A

In Figure 5A, we are interested in comparing MRC1 promoter activity (RLU: % of max Ctrl) between control and 3 other groups: AD8-1, AD8-2, and AD8-3, at 0.2 PU.1 ug DNA (maximum amount).

Comparisons of MRC1 Promoter Activity - Figure 5A

AD8-1	AD8-2	AD8-3
0.003	<0.001	<0.001

p values are from a t test comparing MRC1 promoter activity between control to each group presented in the table above at 0.2 PU.1 ug DNA

Figure 5B

In Figure 5B, we are interested in comparing MRC1 promoter activity (RLU: % of max Ctrl) between control and 6 other groups: WT, Δ Vif, Δ Vpr, Δ Vpu, Δ Nef, and Δ Env, at 0.2 PU.1 ug DNA (maximum amount).

Comparisons of MRC1 Promoter Activity – Figure 5B

WT	dVif	dVpr	dVpu	dNef	dEnv
0.014	0.009	0.013	0.031	0.019	0.007

p values are from a t test comparing MRC1 promoter activity between control to each group presented in the table above at 0.2 PU.1 ug DNA

Figure 5C

In Figure 5C, we are interested in comparing MRC1 promoter activity (RLU: % of max [Tat(-)]) between Tat(-) and 3 other groups: Tat-86, Tat-101, and pNL-A1, at 0.2 PU.1 ug DNA (maximum amount).

Comparisons of MRC1 Promoter Activity – Figure 5C

Tat-86	Tat-101	pNL-A1
0.002	0.001	0.002

p values are from a t test comparing MRC1 promoter activity between Tat(-) to each group presented in the table above at 0.2 PU.1 ug DNA

Figure 5E

In Figure 5E, we are interested in comparing MRC1 promoter activity when Tat101 ug DNA = 0 versus when Tat101 ug DNA = 0.2, in the Ctrl and PU.1-05 groups separately.

Comparisons of MRC1 Promoter Activity – Figure 5E

Ctrl	PU.1-05
0.985	<0.001

p values are from a t test comparing MRC1 promoter activity between 0 and 0.2 Tat ug DNA

in each group in the table above

We are also interested in determining if there is a dose-response relationship between Tat101 ug DNA and MRC1 promoter activity, and how the presence/absence of PU.1 might affect this relationship. We do a linear regression to predict MRC1 promoter activity from Tat101 ug DNA, group (Ctrl vs. PU.1), and an interaction term of group and Tat101 ug DNA. The interaction term tells us if the effect of Tat101 ug DNA on MRC1 promoter activity is different between Ctrl and PU.1.

Linear Regression to predict MRC1 promoter activity - Figure 5E

	Estimate	Std. Error	t value	p value
Intercept	96.743	2.898	33.387	<0.001
Tat101	-162.366	27.628	-5.877	<0.001
Group: Ctrl	-83.341	4.098	-20.338	<0.001
Interaction	167.832	39.072	4.295	<0.001

When Tat101 = 0, the value of MRC1 promoter activity, on average, is 96.743 in the PU.1 group and 13.402 in the Ctrl group. The value of MRC1 promoter activity when Tat101 = 0 is significantly different between Ctrl and PU.1.

The interaction term is significant, indicating that the effect of Tat101 ug DNA on MRC1 promoter activity is different between Ctrl and PU.1 groups. The slope of the line for each group is significantly different from each other.

For PU.1, the slope is -162.366 (95% CI: [-217.497, -107.235]), and for Ctrl, the slope is 5.466 (95% CI: [-49.665, 60.596]). The effect of Tat101 on MRC1 promoter activity is significant for the PU.1 group only.

Figure 6

Figure 6C

In Figure 6C, we are interested in comparing log₁₀ LTR promoter activity between Tat wt and C22G, at 0.2 Tat ug DNA (maximum amount).

We use a t test to compare the log₁₀-LTR promoter activity between these 2 groups at 0.2 Tat ug DNA. The p value for this test is <0.001.

Figure 6D

In Figure 6D, we are interested in comparing log₁₀ MRC1 promoter activity between Tat wt and Tat C22G, at 0.2 Tat ug DNA (maximum amount).

We use a t test to compare the log₁₀ MRC1 promoter activity between these 2 groups at 0.2 Tat ug DNA. The p value for this test is 0.562.

We are also interested in comparing log₁₀ MRC1 promoter activity when Tat ug DNA = 0 versus when Tat ug DNA = 0.2, in the Tat wt and Tat C22G groups separately.

Comparisons of log₁₀ MRC1 promoter activity – Figure 6D

Tat wt	Tat C22G
<0.001	<0.001

p values are from a t test comparing MRC1 promoter activity between 0 and 0.2 Tat ug DNA

in each group in the table above

Promoter activity was log₁₀ transformed

Finally, we are interested in determining if there is a dose-response relationship between Tat ug DNA and log₁₀ MRC1 promoter activity, and how the Tat wt vs. Tat C22G affect this relationship. We do a linear regression to predict log₁₀ MRC1 promoter activity from Tat ug DNA, group (Tat wt vs. Tat C22G), and an interaction term of group and Tat ug DNA. The interaction term tells us if the effect of Tat ug DNA on log₁₀ MRC1 promoter activity is different between Tat wt and Tat C22G.

Linear Regression to predict log₁₀ MRC1 promoter activity – Figure 6D

	Estimate	Std. Error	t value	p value
Intercept	4.281	0.031	136.359	<0.001
Tat	-1.416	0.299	-4.732	<0.001
Group: Tat wt	-0.009	0.044	-0.199	0.843
Interaction	-0.139	0.423	-0.329	0.743

When Tat = 0, the value of log₁₀ MRC1 promoter activity, on average, is 4.281 in the Tat C22G group and 4.272 in the Tat wt group. The value of log₁₀ MRC1 promoter activity when Tat = 0 is not significantly different between the Tat C22G and Tat wt groups.

The interaction term is not significant, indicating that the effect of Tat ug DNA on log₁₀ MRC1 promoter activity is not different between Tat C22G and Tat wt groups. The slope of the line for each group is not significantly different from each other.

For Tat C22G, the slope is -1.416 (95% CI: [-2.011, -0.822]), and for Tat wt, the slope is -1.556 (95% CI: [-2.15, -0.961]). The effect of Tat on log₁₀ MRC1 promoter activity is significant regardless of group.

Figure 7

Figure 7A

In Figure 7A, we are interested in comparing relative MRC1 mRNA levels (hMRC1:GAPDH) between untreated AD8(-) and 5 other groups: untreated AD8(+), Ctrl siRNA AD8(-), Ctrl siRNA AD8(+), PU.1 siRNA AD8(-), and PU.1 siRNA AD8(+).

Comparisons of Relative MRC1 mRNA levels - Figure 7A

	untreated.AD8+	Ctrl siRNA.AD8-	Ctrl siRNA.AD8+	PU.1 siRNA.AD8-	PU.1 siRNA.AD8+
Mean Difference (95% CI)	0.534 (-0.317, 1.386)	0.008 (-0.223, 0.206)	0.393 (-1.967, 1.182)	0.685 (-1.046, -0.323)	0.904 (-0.994, -0.813)
p value	0.082	0.84	0.201	0.022	0.001

p values are from a t test comparing relative MRC1 mRNA levels untreated AD8(-) to each group in the table above

Also shown are differences in means between untreated AD8(-) and each group, along with 95% CI for those differences

Figure 7D

In Figure 7D, we are interested in comparing Gag synthesis (% of Ctrl siRNA) between Ctrl siRNA and PU.1 siRNA.

We use a t test to compare Gag synthesis between these 2 groups. The p value for this test is 0.005.

Figure 7E

In Figure 7E, we are interested in comparing virus release (% total Gag) between Ctrl siRNA and PU.1 siRNA at 4 hours chase (maximum).

We use a t test to compare virus release between these 2 groups. The p value for this test is 0.623.

Figure 8

Figure 8A

In Figure 8A, we are interested in comparing log₁₀ LTR promoter activity (RLU) between pGL3-basic and pGL-LTR-Luc, as well as comparing log₁₀ LTR promoter activity (RLU) between pGL-LTR-Luc and pGL-LTR-Luc+Tat101.

We use a t test to compare log₁₀ LTR promoter activity between these groups. The p value for the test comparing log₁₀ LTR promoter activity between pGL3-basic and pGL-LTR-Luc is 0.001. The p value for the test comparing log₁₀ LTR promoter activity between pGL-LTR-Luc and pGL-LTR-Luc+Tat101 is <0.001.

Figure 8B

In Figure 8B, we are interested in comparing log₁₀ LTR promoter activity (absolute) when PU.1 ug DNA = 0 versus when PU.1 ug DNA = 0.2, in the Ctrl and Tat groups separately.

Comparisons of log₁₀ LTR promoter activity (absolute) - Figure 8B

Ctrl	Tat
<0.001	0.037

p values are from a t test comparing log₁₀ LTR promoter activity (absolute) between 0 and 0.2 PU.1 ug DNA

in each group in the table above. LTR Promoter Activity was log₁₀ transformed

In addition, we do a linear regression to predict log₁₀ LTR promoter activity (absolute) from PU.1 ug DNA, group (Ctrl vs. Tat), and an interaction term of group and PU.1 ug DNA. The interaction term tells us if the effect of PU.1 ug DNA on log₁₀ LTR promoter activity (absolute) is different between Ctrl and Tat.

Linear Regression to predict log₁₀ LTR promoter activity (absolute) - Figure 8B

	Estimate	Std. Error	t value	p value
Intercept	7.453	0.027	273.406	<0.001
PU.1	-0.404	0.260	-1.555	0.123
Group: Ctrl	-1.211	0.039	-31.423	<0.001
Interaction	-0.746	0.368	-2.029	0.045

When PU.1 = 0, the value of log₁₀ LTR promoter activity (absolute), on average, is 7.453 in the Tat group and 6.242 in the Ctrl group. The value of log₁₀ LTR promoter activity when PU.1 = 0 is significantly different between Ctrl and Tat.

The interaction term is significant, indicating that the effect of PU.1 ug DNA on log₁₀ LTR promoter activity is different between Ctrl and Tat groups. The slope of the line for each group is significantly different from each other.

For Tat, the slope is -0.404 (95% CI: [-0.92, 0.112]), and for Ctrl, the slope is -1.15 (95% CI: [-1.666, -0.634]). The effect of PU.1 on log10 LTR promoter activity is significant in the control group only.

Figure 8C

In Figure 8C, we are interested in comparing LTR promoter activity (relative) when PU.1 ug DNA = 0 versus when PU.1 ug DNA = 0.2, in the Ctrl and Tat groups separately.

Comparisons of LTR promoter activity (relative) – Figure 8C

Ctrl	Tat
<0.001	0.001

p values are from a Wilcoxon test comparing LTR promoter activity (relative) between 0 and 0.2 PU.1 ug DNA in each group in the table above

Note a Wilcoxon test was used to compare groups due to outliers present in the data.

Linear Regression to predict LTR promoter activity (relative) - Figure 8C

	Estimate	Std. Error	t value	p value
Intercept	94.571	5.230	18.083	<0.001
PU.1	-87.065	49.864	-1.746	0.084
Group: Ctrl	-6.846	7.396	-0.926	0.357
Interaction	-122.440	70.519	-1.736	0.086

When PU.1 = 0, the value of LTR promoter activity (relative), on average, is 94.571 in the Tat group and 87.725 in the Ctrl group. The value of LTR promoter activity (%) when PU.1 = 0 is not significantly different between Ctrl and Tat.

The interaction term is not significant, indicating that the effect of PU.1 ug DNA on LTR promoter activity (%) is not different between Ctrl and Tat groups. The slope of the line for each group is not significantly different from each other.

For Tat, the slope is -87.065 (95% CI: [-186.099, 11.97]), and for Ctrl, the slope is -209.504, (95% CI: [-308.539, -110.47]). The effect of PU.1 on LTR promoter activity (relative) is significant in the control group only.

Figure 9

Figure 9C

In Figure 9C, we are interested in comparing log10 LTR promoter activity (RLU) when Tat ug DNA = 0 versus when Tat ug DNA = 0.2, in the LTR-Luc and LTR-mTAR groups separately.

Comparisons of log₁₀ LTR promoter activity (RLU) - Figure 9C

LTR-Luc	LTR-mTAR
0.001	0.009

p values are from a t test comparing LTR promoter activity between 0 and 0.2 Tat ug DNA in each group in the table above
RLU values were log₁₀ transformed

Finally, we are interested in determining if there is a dose-response relationship between Tat ug DNA and log₁₀ LTR promoter activity, and how group (LTR-Luc vs. LTR-mTAR) affects this relationship. We do a linear regression to predict log₁₀ LTR promoter activity from Tat ug DNA, group (LTR-Luc vs. LTR-mTAR), and an interaction term of group and Tat ug DNA. The interaction term tells us if the effect of Tat ug DNA on log₁₀ LTR promoter activity is different between groups.

Linear Regression to predict log₁₀ LTR promoter activity - Figure 9C

	Estimate	Std. Error	t value	p value
Intercept	7.106	0.157	45.376	<0.001
Tat	3.219	1.493	2.156	0.043
Group: LTR-mTAR	-0.547	0.221	-2.472	0.023
Interaction	-2.185	2.112	-1.035	0.313

When Tat = 0, the value of log₁₀ LTR promoter activity, on average, is 7.106 in the LTR-Luc group and 6.559 in the LTR-mTAR group. The value of log₁₀ LTR promoter activity when Tat = 0 is significantly different between the LTR-Luc and LTR-mTAR groups.

The interaction term is not significant, indicating that the effect of Tat ug DNA on log₁₀ LTR promoter activity is not different between the LTR-Luc and LTR-mTAR groups. The slope of the line for each group is not significantly different from each other.

For LTR-Luc, the slope is 3.219 (95% CI: [0.104, 6.334]), and for LTR-mTAR, the slope is 1.034, (95% CI: [-2.081, 4.149]). The effect of Tat on log₁₀ LTR promoter activity is significant in the LTR-Luc group only.

Figure 9D

In Figure 9D, we are interested in comparing LTR-mTAR promoter activity (% of no PU.1) between Ctrl and Tat(+), at 0.2 PU.1 ug DNA (maximum amount).

We use a t test to compare the LTR-mTAR promoter activity between these 2 groups at 0.2 PU.1 ug DNA. The p value for this test is 0.034.

We are also interested in comparing LTR-mTAR promoter activity when PU.1 ug DNA = 0 versus when PU.1 ug DNA = 0.2, in the Ctrl and Tat(+) groups separately.

Comparisons of LTR-mTAR promoter activity – Figure 9D

Ctrl	Tat(+)
<0.001	0.006

p values are from a t test comparing LTR-mTAR promoter activity between 0 and 0.2 PU.1 ug DNA in each group in the table above

Finally, we are interested in determining if there is a dose-response relationship between PU.1 ug DNA and LTR-mTAR promoter activity, and how the presence/absence of Tat affect this relationship. We do a linear regression to predict LTR-mTAR promoter activity from PU.1 ug DNA, group (Ctrl vs. Tat(+)), and an interaction term of group and PU.1 ug DNA. The interaction term tells us if the effect of PU.1 ug DNA on LTR-mTAR promoter activity is different between Ctrl and Tat(+).

Linear Regression to predict LTR-mTAR promoter activity - Figure 9D

	Estimate	Std. Error	t value	p value
Intercept	99.485	6.203	16.039	<0.001
PU.1	-154.093	51.452	-2.995	0.004
Group: Ctrl	-10.420	8.772	-1.188	0.240
Interaction	-76.351	72.765	-1.049	0.299

When PU.1 = 0, the value of LTR-mTAR promoter activity, on average, is 99.485 in the Tat(+) group and 89.065 in the Ctrl group. The value of LTR-mTAR promoter activity when PU.1 = 0 is not significantly different between the Tat(+) and Ctrl groups.

The interaction term is not significant, indicating that the effect of PU.1 ug DNA on LTR-mTAR promoter activity is not different between the Tat(+) and Ctrl groups. The slope of the line for each group is not significantly different from each other.

For Tat(+), the slope is -154.093, (95% CI: [-257.438, -50.749]), and for Ctrl, the slope is -230.444, (95% CI: [-333.789, -127.099]). The effect of PU.1 on mTAR-LTR promoter activity is significant regardless of group.

Figure 10

Figure 10B

In figure 10B, we are interested in comparing log₁₀ MRC1-Luc activity between WT and S148A at 0.2 ug PU.1 DNA (maximum amount).

We use a t test to compare the log₁₀ MRC1-Luc activity between these 2 groups at 0.2 PU.1 ug DNA. The p value for this test is 0.069.

We are also interested in comparing log₁₀ MRC1-Luc activity when PU.1 ug DNA = 0 versus when PU.1 ug DNA = 0.2, in the WT and S148A groups separately.

Comparisons of MRC1-Luc activity - Figure 10B

WT	S148A
0.003	0.001

p values are from a t test comparing MRC1-Luc activity between 0 and 0.2 PU.1 ug DNA in each group in the table above

Outcome values are log₁₀ transformed

Finally, we are interested in determining if there is a dose-response relationship between PU.1 ug DNA and log₁₀ MRC1-Luc activity, and how this relationship differs between WT and S148A. We do a linear regression to predict log₁₀ MRC1-Luc activity from PU.1 ug DNA, group (WT vs. S148A), and an interaction term of group and PU.1 ug DNA. The interaction term tells us if the effect of PU.1 ug DNA on log₁₀ MRC1-Luc activity is different between WT and S148A.

Linear Regression to predict log₁₀ MRC1-Luc activity - Figure 10B

	Estimate	Std. Error	t value	p value
Intercept	4.765	0.088	54.031	<0.001
PU.1	3.356	0.841	3.991	0.001
Group: WT	-0.024	0.125	-0.195	0.847
Interaction	-0.288	1.189	-0.242	0.811

When PU.1 = 0, the value of log₁₀ MRC1-Luc activity, on average, is 4.765 in the S148A group and 4.741 in the WT group. The value of log₁₀ MRC1-Luc activity when PU.1 = 0 is not significantly different between the S148A and WT groups.

The interaction term is not significant, indicating that the effect of PU.1 ug DNA on log₁₀ MRC1-Luc activity is not different between the S148A and WT groups. The slope of the line for each group is not significantly different from each other.

For S148A, the slope is 3.356, (95% CI: [1.602, 5.11]), and for WT, the slope is 3.068, (95% CI: [1.314, 4.822]). The effect of PU.1 on log₁₀ MRC1-Luc activity is significant regardless of group.

Figure 10C

In figure 10C, we are interested in comparing log₁₀ LTR-Luc activity between WT and S148A at 0.2 ug PU.1 DNA (maximum amount).

We use a t test to compare the log₁₀ LTR-Luc activity between these 2 groups at 0.2 PU.1 ug DNA. The p value for this test is 0.391.

We are also interested in comparing LTR-Luc activity when PU.1 ug DNA = 0 versus when PU.1 ug DNA = 0.2, in the WT and S148A groups separately.

Comparisons of log₁₀ LTR-Luc activity - Figure 10C

WT	S148A
0.023	0.017

p values are from a t test comparing LTR-Luc activity between 0 and 0.2 PU.1 ug DNA in each group in the table above

Outcome values are log₁₀ transformed

Finally, we are interested in determining if there is a dose-response relationship between PU.1 ug DNA and log₁₀ LTR-Luc activity, and how this relationship differs between WT and S148A. We do a linear regression to predict log₁₀ LTR-Luc activity from PU.1 ug DNA, group (WT vs. S148A), and an interaction term of group and PU.1 ug DNA. The interaction term tells us if the effect of PU.1 ug DNA on log₁₀ LTR-Luc activity is different between WT and S148A.

Linear Regression to predict LTR-Luc activity - Figure 10C

	Estimate	Std. Error	t value	p value
Intercept	6.518	0.039	165.415	<0.001
PU.1	-1.106	0.376	-2.945	0.008
Group: WT	0.028	0.056	0.495	0.626
Interaction	0.192	0.531	0.361	0.722

When PU.1 = 0, the value of log₁₀ LTR-Luc activity, on average, is 6.518 in the S148A group and 6.546 in the WT group. The value of log₁₀ LTR-Luc activity when PU.1 = 0 is not significantly different between the S148A and WT groups.

The interaction term is not significant, indicating that the effect of PU.1 ug DNA on log₁₀ LTR-Luc activity is not different between the S148A and WT groups. The slope of the line for each group is not significantly different from each other.

For S148A, the slope is -1.106, (95% CI: [-1.89, -0.323]), and for WT, the slope is -0.915, (95% CI: [-1.698, -0.131]). The effect of PU.1 on log₁₀ LTR-Luc activity is significant regardless of group.

Figure 10D

In figure 10D, we are interested in comparing MRC1-Luc activity between WT and S148A at 0.2 ug Tat101 DNA (maximum amount).

We use a t test to compare the MRC1-Luc activity between these 2 groups at 0.2 Tat101 ug DNA. The p value for this test is 0.954.

We are also interested in comparing MRC1-Luc activity when Tat101 ug DNA = 0 versus when Tat101 ug DNA = 0.2, in the WT and S148A groups separately.

Comparisons of MRC1-Luc activity - Figure 10D

wt	S148A
<0.001	0.004

p values are from a t test comparing MRC1-Luc activity between 0 and 0.2 Tat101 ug DNA in each group in the table above

Finally, we are interested in determining if there is a dose-response relationship between Tat101 ug DNA and MRC1-Luc activity, and how this relationship differs between WT and S148A. We do a linear regression to predict MRC1-Luc activity from Tat101 ug DNA, group (WT vs. S148A), and an interaction term of group and Tat101 ug DNA. The interaction term tells us if the effect of Tat101 ug DNA on MRC1-Luc activity is different between WT and S148A.

Linear Regression to predict MRC1-Luc activity - Figure 10D

	Estimate	Std. Error	t value	p value
Intercept	106.648	7.976	13.370	<0.001
Tat101	-263.191	66.164	-3.978	<0.001
Group: WT	-4.192	11.280	-0.372	0.713
Interaction	8.588	93.570	0.092	0.927

When Tat101 = 0, the value of MRC1-Luc activity, on average, is 106.648 in the S148A group and 102.456 in the WT group. The value of MRC1-Luc activity when Tat101 = 0 is not significantly different between the S148A and WT groups.

The interaction term is not significant, indicating that the effect of Tat101 ug DNA on MRC1-Luc activity is not different between the S148A and WT groups. The slope of the line for each group is not significantly different from each other.

For S148A, the slope is -263.191, (95% CI: [-397.963, -128.419]), and for WT, the slope is -254.603, (95% CI: [-389.375, -119.83]). The effect of Tat101 on MRC1-Luc activity is significant regardless of group.

