Figures and Preliminary Studies:

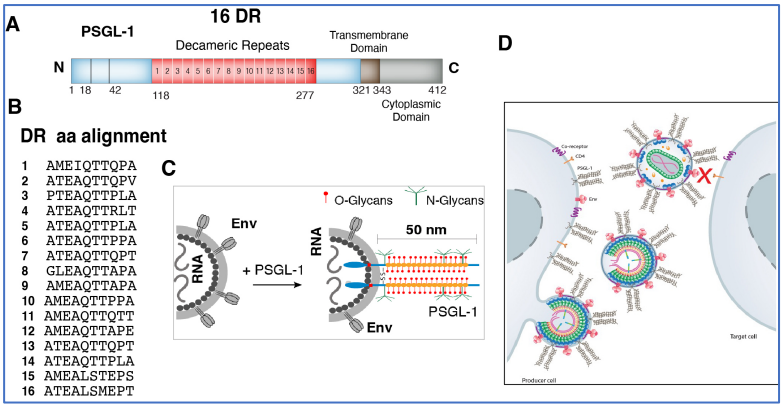

Fig. 1. PSGL-1 and model of steric hindrance. (**A**) Schematic of PSGL-1 showing the extracellular domain, transmembrane domain, and the intracellular cytoplasmic domain. The location of the 16 decameric repeats (DR) is shown. The AA sequence of individual DRs is shown in (**B**). (**C**) **Virion incorporation of PSGL-1.** PSGL-1 is incorporated into virus particles during HIV assemble. A fully extended PSGL-1 can reach 50-60 nm on virion particles. (**D**) **Steric hinderance model**: PSGL-1 on viral particles are highly extended that can sterically hinder the HIV Env binding to target cells.

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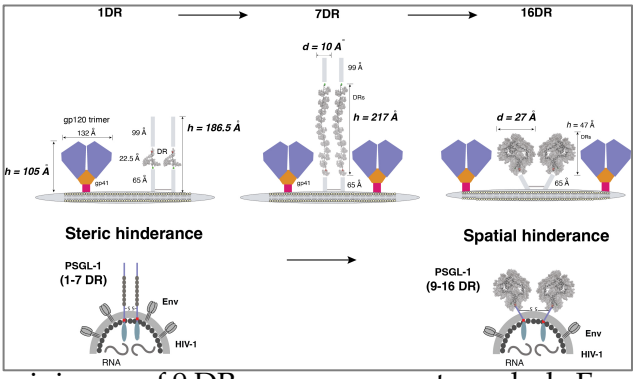
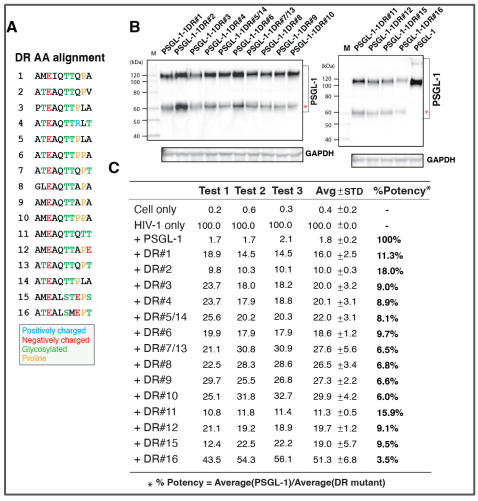


Fig. 2. Molecular modeling of PSGL-1's antiviral mechanisms. PSGL-1 inhibits HIV infectivity through two distinct mechanisms: **1. Steric hindrance**, where PSGL-1 is incorporated into virions, obstructing their attachment to target cells. In steric hinderance, a single DR is sufficient to maintain basal activity. The estimated height of the fully-extended extracellular domain of a single DR mutant is approximately **187 Å**, which surpasses that of the Env trimer (approximately **105 Å**) on the virion membrane. **2. Spatial hindrance**, wherein PSGL-1 prevents the incorporation of HIV Env into virion particles. In spatial hinderance, a minimum of 9 DRs are necessary to exclude Env. PSGL-1 mutants with 7 or fewer DRs adopt an extended "rod-like" structure, whereas those with 9 or more DRs collapse into a "coil-like" structure, enhancing their ability to exclude Env spatially.



	Test 1	Test 2	Test 3	Avg ±STD	%Potency*
Cell only	0.2	0.6	0.3	0.4 ±0.2	-
HIV-1 only	100.0	100.0	100.0	100.0 ±0.0	-
+ PSGL-1	1.7	1.7	2.1	1.8 ±0.2	100%
+ DR#1	18.9	14.5	14.5	16.0 ±2.5	11.3%
+ DR#2	9.8	10.3	10.1	10.0 ±0.3	18.0%
+ DR#3	23.7	18.0	18.2	20.0 ±3.2	9.0%
+ DR#4	23.7	17.9	18.8	20.1 ±3.1	8.9%
+ DR#5/14	25.6	20.2	20.3	22.0 ±3.1	8.1%
+ DR#6	19.9	17.9	17.9	18.6 ±1.2	9.7%
+ DR#7/13	21.1	30.8	30.9	27.6 ±5.6	6.5%
+ DR#8	22.5	28.3	28.6	26.5 ±3.4	6.8%
+ DR#9	29.7	25.5	26.8	27.3 ±2.2	6.6%
+ DR#10	25.1	31.8	32.7	29.9 ±4.2	6.0%
+ DR#11	10.8	11.8	11.4	11.3 ±0.5	15.9%
+ DR#12	21.1	19.2	18.9	19.7 ±1.2	9.1%
+ DR#15	12.4	22.5	22.2	19.0 ±5.7	9.5%
+ DR#16	43.5	54.3	56.1	51.3 ±6.8	3.5%

* % Potency = Average(PSGL-1)/Average(DR mutant)

Fig. 3. A single DR possesses basal antiviral activities. (**A**) AA sequence alignment of the 16 single DRs of PSGL-1. (**B**) Western blot detection of PSGL-1 DR mutant proteins. (**C**) PSGL-1 (full-length) or its single DR mutants (#1 to #16) (200 ng) were cotransfected with HIV-1 DNA (1000 ng) into HEK293T cells. Virions were harvested and used to infect an HIV-1 Rev-dependent indicator cell, Rev-A3R5-GFP. Virion infectivity was quantified by the relative % of GFP+ cells. "HIV-1 only" was used as 100% infection. To calculate relative antiviral potency, "PSGL-1" was assigned as 100%, and the relative potency was calculated as "Average (PSGL-1)/Average (DR mutant %).

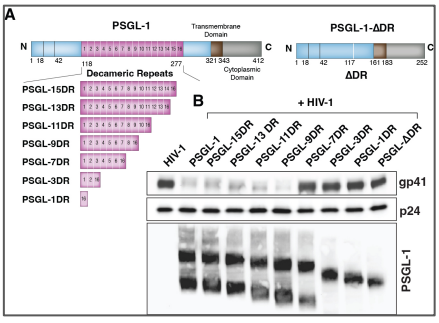


Fig. 4. A minimum of 9 DRs is required for blocking HIV Env incorporation. (**A**) Schematic diagram showing sequential DR deletions in PSGL-1 DR mutants. (**B**) Western blot quantification of HIV gp41 levels in virions produced from cells co-transfected with full-length PSGL-1 DNAs or its DR deletion mutants DNA. Virion p24 was also probed and used for normalizing levels of particles. Normalized levels of gp41 are shown as fold change. Levels of virion PSGL-1 were also quantified.