Supporting Information

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	chronic clade B		clade B T/F			chronic clade C		clade C infant T/F			clade C T/F	chronic clade AE	nonhuman retroviruses	
	MN tier 1A X4	YU2 tier 1B R5	CH40 tier 2 R5	CH77 tier 2 R5	CH058 tier 2 R5	MW965 tier 1 R5	DU156.12 tier 2 R5	BF1677 tier 2 R5	BF329 tier 2 R5	BF942 tier 2 R5	1086C tier 2 R5	CM235 tier 2 R5	SVA.MLV	SIV mac251
HIV- milk														
1*	< 3	3	<3	10	3	7	4	5	5	7		3	<3	3
2	< 3	3	15	26	9	9	5	8	8	9		8	<3	10
3	3	3	25	27	14	10	8	<3	3	4		6	<3	8
4	< 3	5	17	30	13	12	27	3	3	4		4	<3	6
5	< 3	<3	9	15	12	7	9	4	4	5		2	<3	<3
6	< 3	< 3	6	5	5	6	9	<3	<3	<3		<3	<3	<3
7	< 3	3	<3	10	9	3	8	<3	3	<3		<3	<3	<3
8	< 3	3	5	15	5	3	3	<3	3	<3		<3	<3	<3
9	< 3	<3	6	9	5	7	24	<3	5	<3		3	3	<3
10			5	15	6	3	20	3	3	<3		<3	8	<3
Purified protein														
TNC#	130	158	122	118	116	120	110	108	110	100	82	113	109	>180
lactoferrin	>300		>300		>300	>300	>300	>300	>300				>300	
Mucin-1	>300		>300		>300	>300	>300	>300	>300				>300	

ID_{50} 3-5 or IC_{50} 150-180
ID_{50} 5-10 or IC_{50} 110-150
ID_{50} >10 or IC_{50} <110
Not done

* = numbers indicate inhibitory dilution 50% (ID_{50})
# = inhibitory concentration 50% (IC_{50}, µg/ml)

Fig. S1. Broad-spectrum HIV-1–neutralizing activity of breast milk of uninfected women and purified milk proteins. Neutralization activity of breast milk of uninfected women and breast milk proteins TNC, lactoferrin, and mucin-1 in the TZM-bl reporter cell assay, reported as ID_{50} or IC_{50}, respectively.

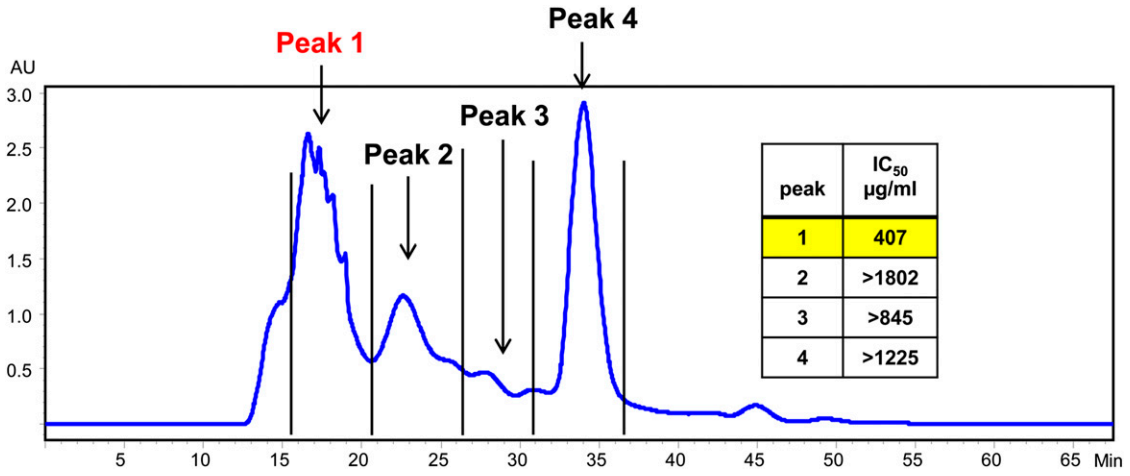


Fig. S2. Size-exclusion fractionation of breast milk reveals that HIV-1–neutralizing activity is solely contained in the high molecular mass fraction. Breast milk fractions (peaks 1–4) were tested for neutralization against the chronic clade C HIV-1 variant C.Du156 (tier 2 neutralization sensitivity) in the TZM-bl neutralization assay. Only the highest molecular mass fraction (peak 1, >500 kDa) had detectable neutralizing activity, with an inhibitory concentration 50% (IC_{50}) of 407 µg/mL. The IC_{50} of each size-fractionated protein peak is listed in the table.

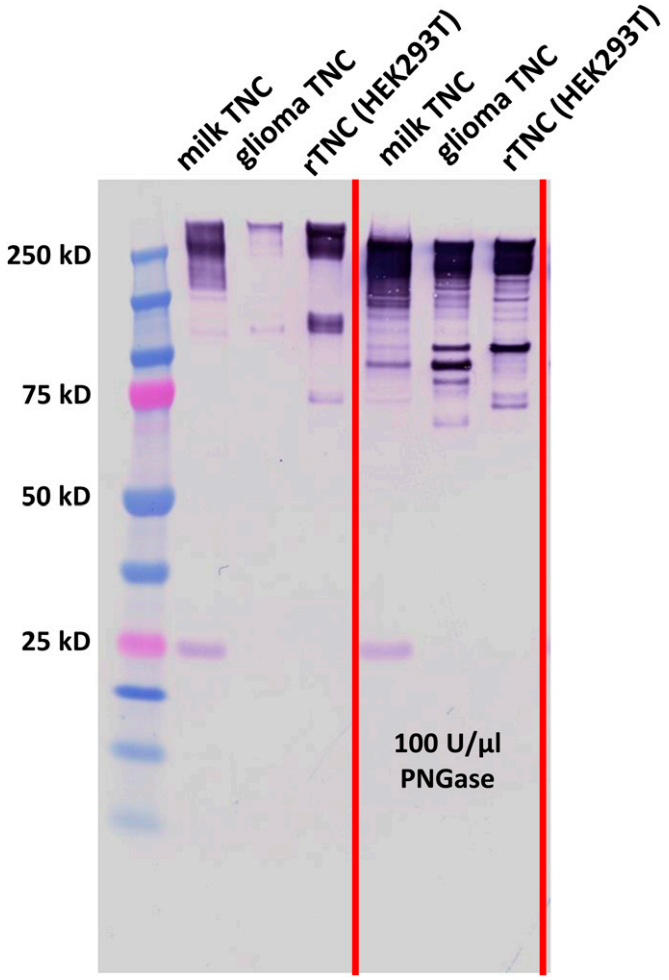


Fig. S3. Distinct N-linked glycosylation pattern of Tenascin-C (TNC) produced in different cell lines. TNC purified from breast milk, purified from a glioma cell line (Millipore), and recombinantly produced by HEK293T cells have distinct banding patterns on an anti-TNC Western blot after deglycosylation with 100 U/µL PGNase (*Right*).

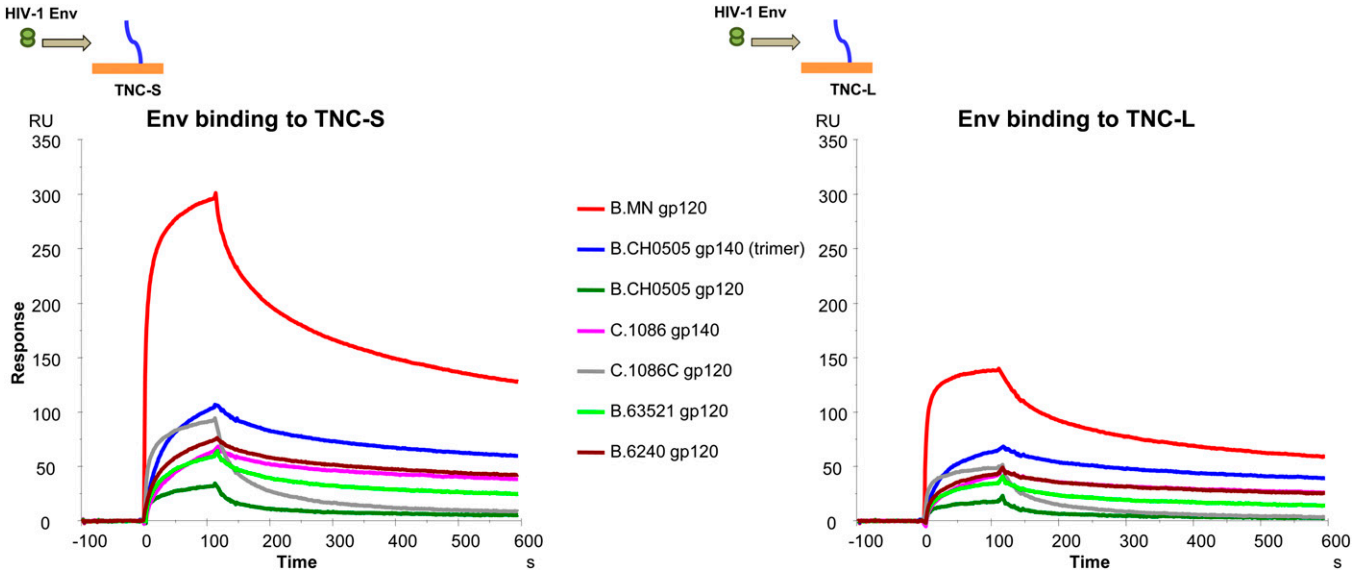
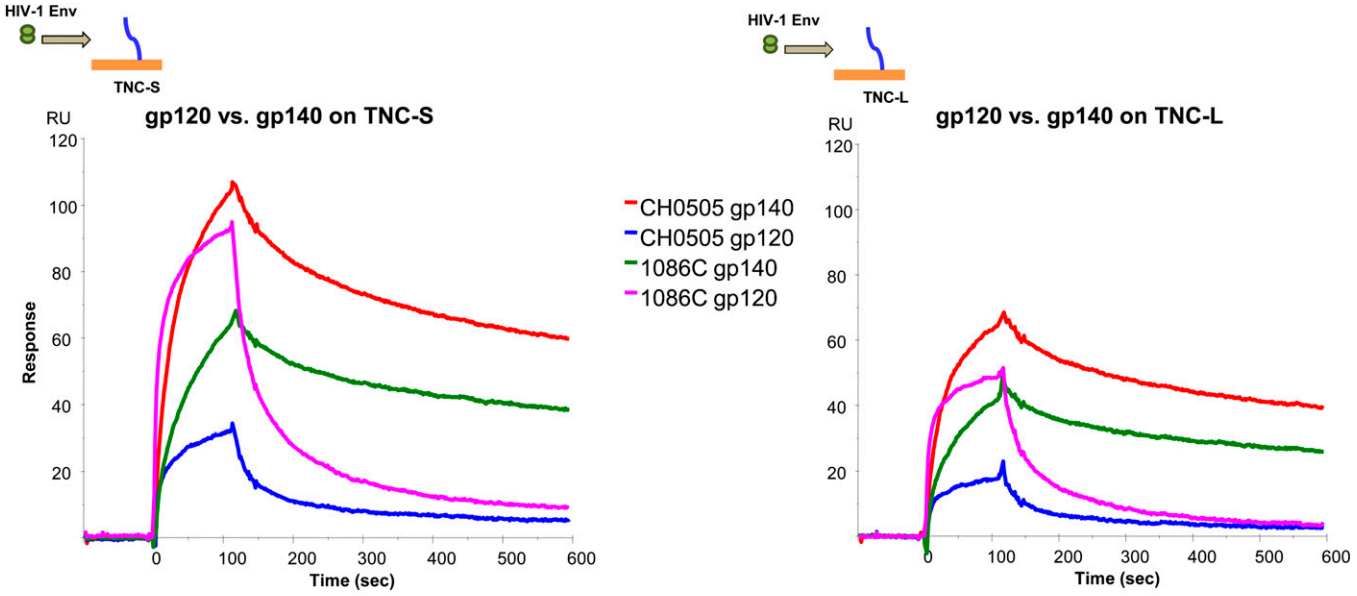


Fig. S4. Spliced short (TNC-S) and long (TNC-L) isoforms of TNC bind to HIV-1 Envelope (Env) gp120 and gp140 proteins. Recombinant TNC-L and TNC-S were covalently coupled to the surface plasmon resonance (SPR) chip, and HIV-1 Env gp120 and gp140 were flowed over the chip. TNC isoforms bound to both clade B and C and consensus Env gp120 and gp140 proteins, including the purified gp140 trimer of the transmitted/founder (T/F) virus CH0505. TNC-S and TNC-L bound with similar affinity to B.MN gp120 (54.8 and 58.2 nM, respectively).



Env	Dissociation rate constant (k_d, s^{-1} x10^{-3})
CH0505 gp120	4.5
CH0505 gp140	1.56
1086 gp120	9.1
1086 gp140	1.55

Env	Dissociation rate constant (k_d, s^{-1} x10^{-3})
CH0505 gp120	5.97
CH0505 gp140	1.1
1086 gp120	7.1
1086 gp140	1.2

Fig. S5. Binding of TNC to Env gp140 has a slower off-rate than the binding of TNC to Env gp120. Recombinant TNC-L and TNC-S were covalently coupled to the SPR chip, and HIV-1 Env gp120 and gp140 were flowed over the chip. TNC isoforms bound to both gp120 and gp140 proteins of the T/F HIV-1 variants B. CH0505 and C.1086, but the binding to gp140 proteins had a slower off-rate.

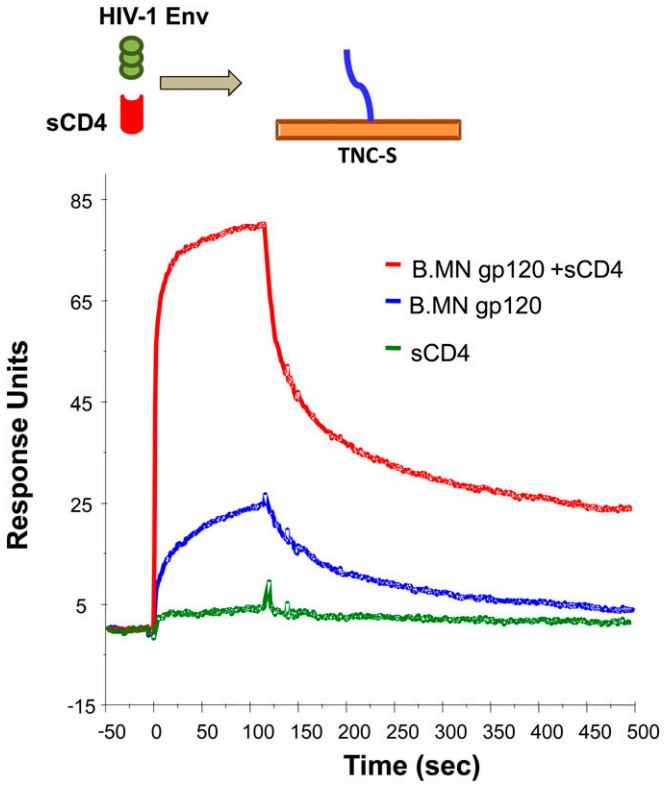


Fig. S6. CD4 binding to TNC does not account for the increased binding of soluble CD4-preincubated gp120 than gp120 alone to TNC. Recombinant TNC-S was covalently coupled to the SPR chip, and HIV-1 B.MN gp120 was flowed over the chip both before and after soluble CD4 preincubation. As a control, soluble CD4 alone was flowed over the TNC-S chip and no binding was detected.

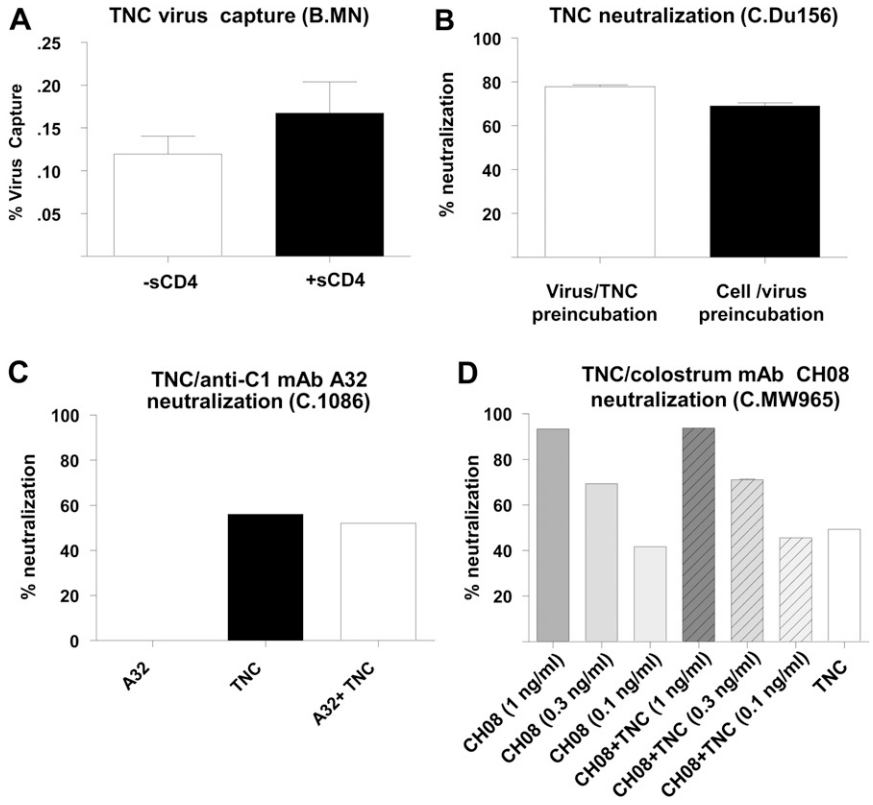


Fig. S7. Env CD4 triggering mildly increases TNC HIV-1 virion capture but not neutralization, and TNC does not antagonize the neutralizing activity of an anti-gp120 CD4-inducible mAb isolated from colostrum. (*A*) Preincubation of HIV-1 B.MN virions with soluble CD4 increases the efficiency of TNC virion capture by ~1.5-fold. (*B*) Preincubation of HIV-1 virions (B.Du156) with TZM-bl cells for 10 min on ice to allow virion–CD4 interaction before addition of TNC does not enhance the neutralizing potency of TNC. Graphs represent data from two assays performed in duplicate; lines indicate SD. (*C*) Preincubation of T/F HIV C.1086 virions with 100 μg/mL anti-C1 Env mAb A32, which is known to induce a conformational change of the HIV-1 Env similar to that of CD4 binding, for 1 h before the addition of TNC (200 μg/mL) and TZM-bl cells does not enhance TNC neutralization. (*D*) TNC does not antagonize the neutralizing potency of the CD4-inducible, colostrum mAb CH08 against HIV-1 C.MW965 at a range of concentrations performed in duplicate.