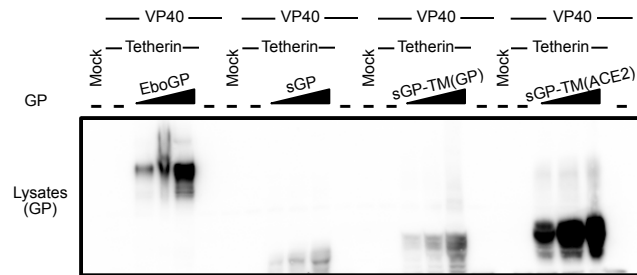


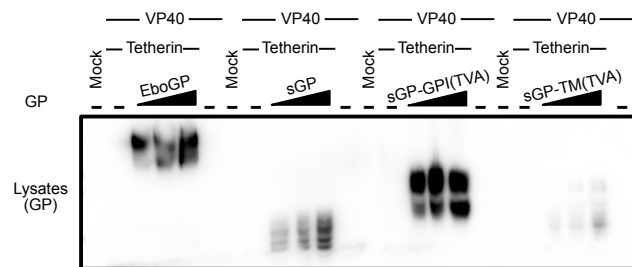
MSD and N-terminal Sequences

EboGP	1912	O G D N D N W W T G W R O W I P A G I G V T G V V I A V I A L F C I C K F V F P	
		CAGGGGGACAATGACAAATG GTGGACAGGATGGAGACAAT GGATACCGGCAGGTATTGGA GTTACAGGCGTGTAAATGC AGTTATCGCTTTATTCTGTA TATGCAAAATTTGCTTTCCG	Ectodomain EboGP Transmembrane Domain Cytoplasmic Tail
	2032	R F E G K P I P N P L L G L D S T R T G H H H H H H *	
		CGGTTGGAAGGTAAGCCTAT CCCTAACCGCTCTCTCGGTC TCGATTCTACGGGTACCGGT CATCATCACCATCACCATTG A	V5-His tag
SGP	946	K T S V V R V R R E L L P T Q G P T Q Q L K T T K S W L Q K I P L Q W F K C T V	
		AAAACATCAGTGGTCTCTAG CCAGCGCGAATCTCTCCGA CCCAGGACCAACACAACAA CTGAAGACCACAAAATCATG GCTTCAGAAAATCCTCTGC AATGGTTCAAGTGCACAGTC	SGP Furin Cut Site Delta Peptide
	1066	K E G K L O C R I *	
		AAGGAAGGGAAGCTGCAGTG TCGCATCTAA	
SGP-TM (GP)	946	K T S V V S R O G D N D N W W T G W R O W I P A G I G V T G V V I A V I A L F C	
		AAAACATCAGTGGTCTCTAG ACAGGGGACAATGACAATT GGTGGACAGGATGGAGACAA TGGATACCGGCAGGTATTGG AGTTACAGGCGTGTAAATG CAGTTATCGCTTTATTCTGT	SGP XbaI EboGP Ectodomain EboGP Transmembrane Domain
	1066	I C K F V F *	
		ATACCAATTTGCTTTA G	EboGP Cytoplasmic Tail
SGP-TM (ACE2)	946	K T S V V S R O P T L G P P N O P P V S I W L I V F G V V M G V I V V G I V I L	
		AAAACATCAGTGGTCTCTAG ACAGCCAACACTTGGACCTC CTAACCAAGCCCTCTTTCC ATATGGCTGATTGTTTGG AGTTGTGATGGGAGTGATAG GGTTGGCATTGTCTATCCTG	SGP XbaI ACE2 Ectodomain ACE2 Transmembrane Domain
	1066	I F T G I R D R K K K N K A R S G E N P Y A S I D I S K G E N N P G F Q N T D D	
		ATCTTCACTGGGATCAGAGA TCGGAAGAAGAAAATAAAG CAAGAAGTGGAGAAAATCCT TATGCCTCCATCGATATTAG CAAAGGAGAAAATAATCCAG GATTCCAAAACACTGATGAT	ACE2 Cytoplasmic Tail
	1186	V Q T S F *	
		GTTCAGACCTCCTTTAG	
SGP-TM (TVA)	946	K T S V V L E P T D N G T E A P T V P A P G R A L P A R N H G R M W M L I T A V	
		AAAACATCAGTGGTCTCTAG GCCCACGGACAACGGCACAG AGGCTCCCACTGTCCCTGC TCCTGGACGTGCTCTGCCAGC CAGGAATCACGGCCGCATGT GGATGCTGATCACTGCAGTG	SGP XhoI TVA Ectodomain
	1066	L L C C L V A V G G I A A W G K S K A K S R S D I F S L E S A S K E L L V P D K	
		CTCCTGTGCTGCTCTGATAG TGTGGGTGGTATCGCTGCAT GGGGGAAGTCCAAAGCAA AAGCAGGTCTGACATCTTCA TCTTGAAGCGCATCCAAGG AGCTGCTGGTGCCTGACAAG	TVA Transmembrane Domain TVA Cytoplasmic Tail
	1186	S Q A D L F S *	
		AGCCAGGCAGACTTGTCTC CTGA	
SGP-GPI (TVA)	946	K T S V V S R P T D N G T E A P T V P A P G R A L P A R N H G R M W M L I T A G	
		AAAACATCAGTGGTCTCTAG ACCCACGGACAACGGCACAG AGGCTCCCACTGTCCCTGC TCCTGGACGTGCTCTGCCAGC CAGGAATCACGGCCGCATGT GGATGCTGATCACTGCAGGG	SGP XbaI TVA Ectodomain and GPI anchor signal sequence
	1186	I F C C E L V R W D *	
		ATCTTTTGCTGTGAGCTGDT GAGATGGGACTGA	
Tetherin	1	M A S T S Y D Y C R V P M E D G D K R C K L L L G I G I L V L L I I V I L G V P	
		ATGGCATCTACTTCGTATGA CTATTGCAGAGTGCCCATGG AAGACGGGATAAGCGCTGT AAGCTTCTGCTGGGGATAGG AATTCTGGTGTCTCTGATCA TCGTGATTTCTGGGGGTGCCC	Tetherin Cytoplasmic Tail Tetherin Transmembrane Domain
	121	L I I F T I K A N S	
		TTGATTATCTTCACCATCAA GGCCAACAGC	
mtf1l-Tetherin	1	M D T Y R Y I M D Q A R S A F S N L F G G E P L S Y T R F S L A R Q V D G D N S	
		ATGGATACATATCGATACAT TATGGATCAAGCCAGATCAG CATTCTCTAAGTGTGTTGGT GGGGAACCATTTGTCATACAC CCGGTTTAGCTTGCTCGGC AAGTAGATGGAGATAACAGT	AUI tag mtf1l Cytoplasmic Tail
	121	H V E M K L A A D E E E N A D N N M K A S V R K P K R F N G R L C F A A I A L V	
		CATGTGGAGATGAACTGGC TGCAGATGAAGAAGAAAATG CCGACAATAACATGAAGGCT AGTGTCAGAAAACCAAGAG GTTTAATGGAAGACTCTGCT TTGCAGCTATTGCACTAGTC	
	241	I F F L I G F M S G Y L G T I K A N S	
		ATTTTCTTCTTGATTGGATT CATGAGTGGCTACCTGGGCA CCATCAAGGCCAACAGC	mtf1l Transmembrane Domain

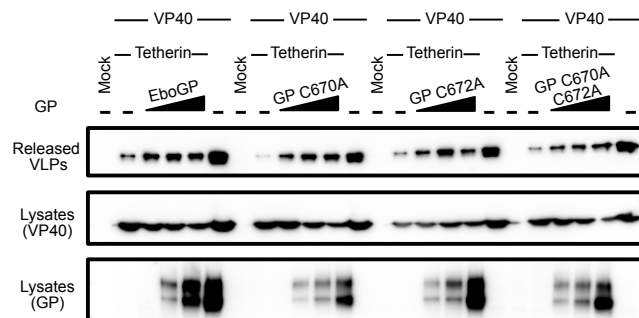
Supplemental Figure 1. A nucleotide sequence list comparing the unique C-terminal or N-terminal domains for each construct used in this study. The amino acid translation is given above each sequence and annotations are indicated below each sequence.



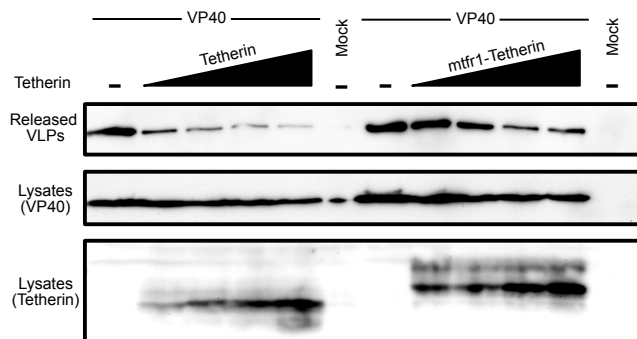
Supplemental Figure 2. SDS-PAGE immunoblot of 293T cell lysates analyzing expression of the glycoproteins used in the budding assay from Figure 1C. The immunoblot of the cell lysates from Figure 1C was stripped and reprobed with the R12 antibody to detect verify expression of the constructs in the budding assay.



Supplemental Figure 3. Immunoblot analyzing glycoprotein expression in the 293T cell lysates from the budding assay in Figure 1D. The western blot from the budding assay in Figure 1D was stripped and re-probed with the R12 antibody to confirm expression of the new constructs.



Supplemental Figure 4. A VP40 VLP budding assay assessing the ability of EboGP to antagonize tetherin with either one or both membrane proximal cysteines modified to an alanine. *Top Panel:* An immunoblot depicting purified VLPs released by each of the glycoproteins, suggesting that modification of the cysteine residues at 670 and 672 do not affect VLP release. *Middle Panel:* Cellular lysates were also analyzed by immunoblot to verify the expression of VP40. *Bottom Panel:* The middle panel immunoblot was stripped and reprobed with the R12 antibody to detect glycoprotein expression in the cellular lysates.



Supplemental Figure 5. A VP40 VLP budding assay comparing the ability of tetherin and mtfr1-tetherin to prevent release of VLPs into the supernatant. *Top Panel:* VLPs analyzed by immunoblot showing the effectiveness of both tetherin and mtfr1-tetherin in retaining budded particles. *Middle Panel:* An immunoblot showing the expression of VP40 in the corresponding cellular lysates. *Bottom Panel:* The immunoblot from the middle panel was reprobed for tetherin expression.