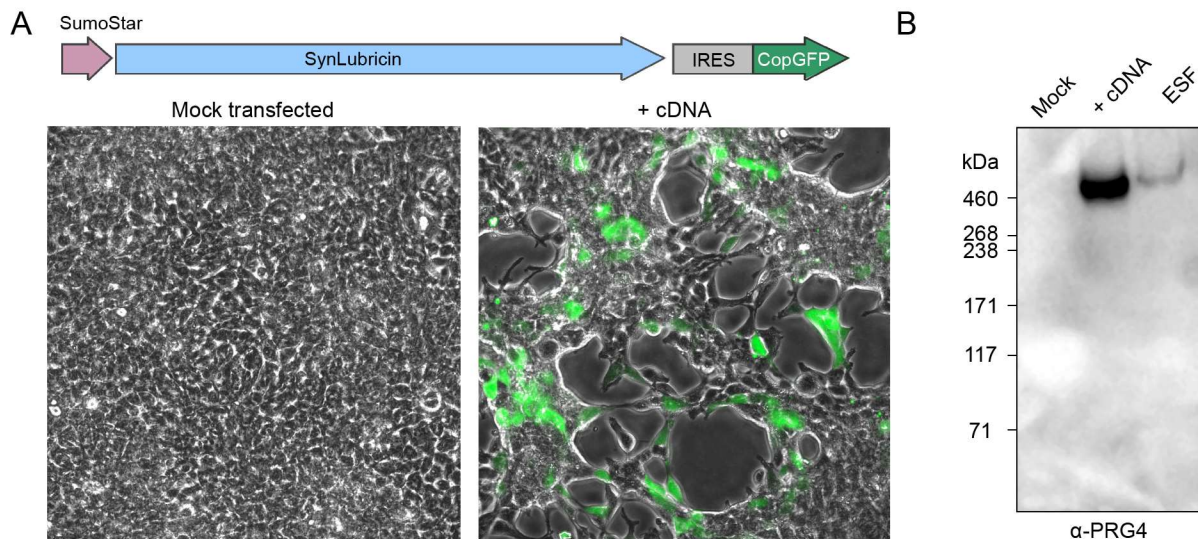


STABLE RECOMBINANT PRODUCTION OF CODON-SCRAMBLED LUBRICIN AND MUCIN IN HUMAN CELLS

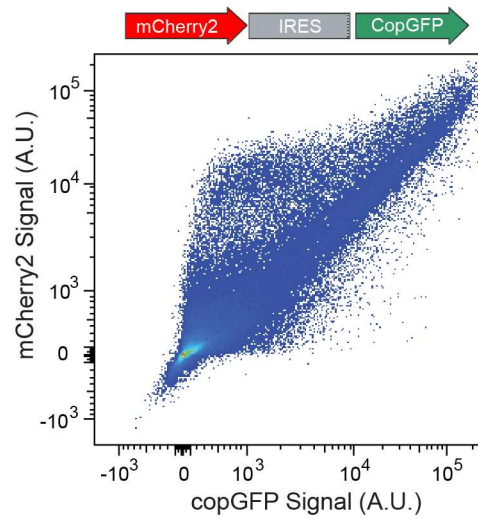
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SUPPLEMENTAL FIGURES

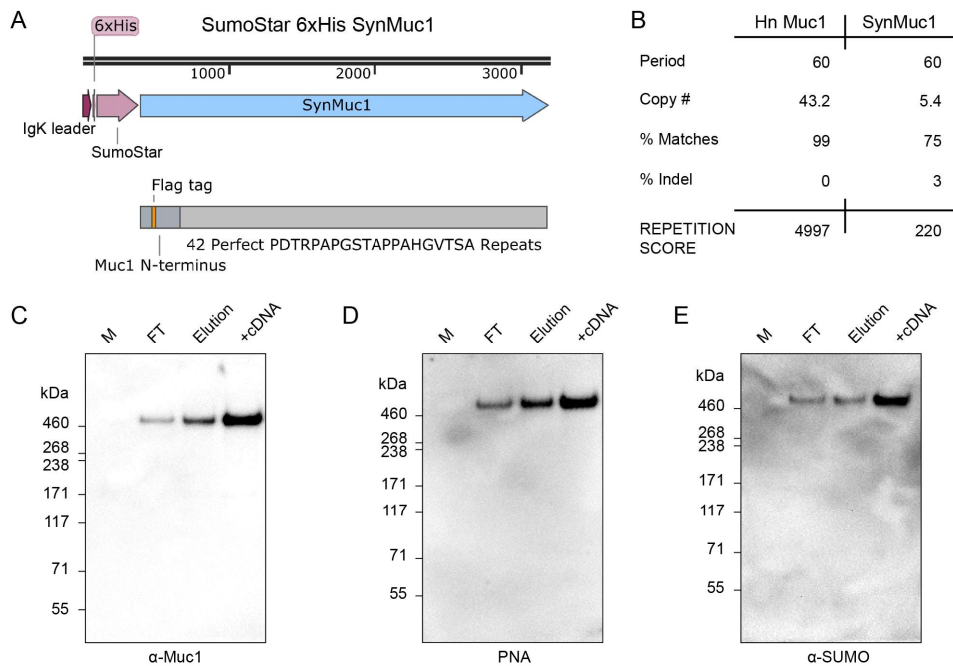


Supplemental Figure 1: Transient expression of SynLubricin altered adherent cell morphology. **A)** Morphology of 293-T cells mock transfected or transfected with cDNA for bicistronic SynLubricin IRES copGFP. Images shown are a merged overlay of phase contrast and fluorescence micrographs. Note the inhibition of cell-cell adhesion near cells expressing high levels of the copGFP reporter. **B)** Western blot of equine synovial fluid (ESF) and media supernatant from mock-transfected and SynLubricin-transfected cells probed with MABT401 antibody against PRG4 tandem repeats.



Supplemental Figure 2: Validation of new transposon-based gene delivery vector.

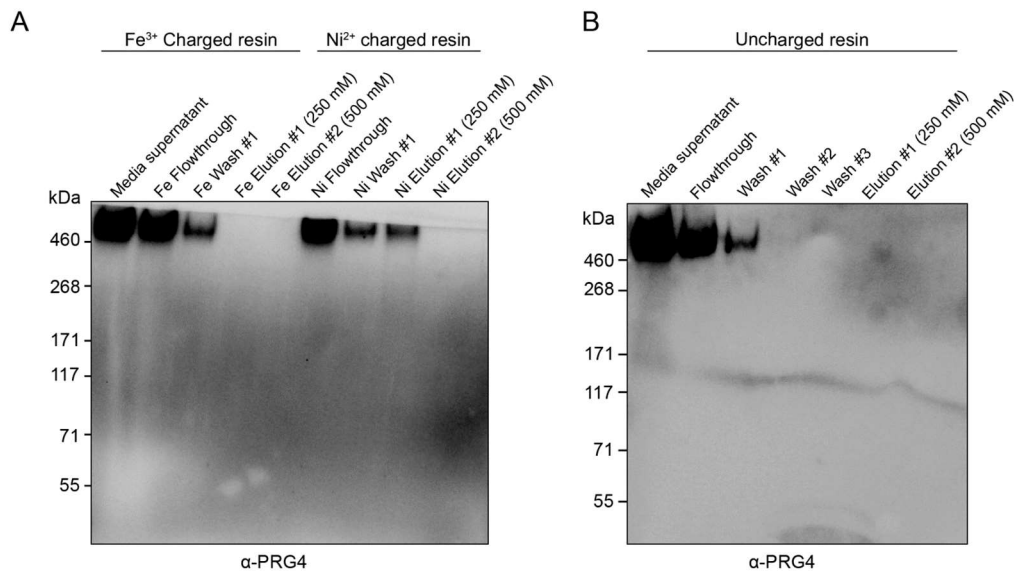
Flow cytometry results showing correlation of levels of mCherry2 and the copGFP reporter.



Supplemental Figure 3: Application of the codon-scrambling strategy for Muc1. A)

Schematic of SynMuc1 with codon-scrambled tandem repeats. **B)** Calculated repetition

score for the nucleotides encoding the tandem repeats of human Muc1 and SynMuc1. **C)** Western blot of media supernatant from 293-F cells transfected with SynMuc1 cDNA (+cDNA) or non-transfected cells (M), Ni-NTA resin flow through from His-affinity purification (FT), and eluted protein (Elution) probed with a Muc1 antibody. **D)** PNA-lectin blot of C. **E)** Western blot of C, probed with a SUMO antibody.



Supplemental Figure 4: SynLubricin has low affinity for immobilized-metal-affinity-chromatography (IMAC) resin. A) Western blot of media supernatant and the IMAC purification flow throughs, washes, and eluted fractions from Fe³⁺ and Ni²⁺ loaded nitrotriactic acid (NTA) resins. Elutions were performed at the indicated NaCl concentration. No non-specific binding of sialic acids to multivalent Fe³⁺ was observed. **B)** Western blot of flow through, wash, and eluted fractions from uncharged NTA resin.

Human PRG4A RepeatsAmino acid composition

Ala (A)	58	11.4%
Arg (R)	0	0.0%
Asn (N)	1	0.2%
Asp (D)	2	0.4%
Cys (C)	0	0.0%
Gln (Q)	0	0.0%
Glu (E)	48	9.4%
Gly (G)	4	0.8%
His (H)	0	0.0%
Ile (I)	1	0.2%
Leu (L)	7	1.4%
Lys (K)	69	13.6%
Met (M)	0	0.0%
Phe (F)	0	0.0%
Pro (P)	155	30.5%
Ser (S)	13	2.6%
Thr (T)	150	29.5%
Trp (W)	0	0.0%
Tyr (Y)	0	0.0%
Val (V)	0	0.0%

SynLubricin RepeatsAmino acid composition

Ala (A)	59	12.5%
Arg (R)	0	0.0%
Asn (N)	0	0.0%
Asp (D)	0	0.0%
Cys (C)	0	0.0%
Gln (Q)	0	0.0%
Glu (E)	59	12.5%
Gly (G)	0	0.0%
His (H)	0	0.0%
Ile (I)	0	0.0%
Leu (L)	0	0.0%
Lys (K)	59	12.5%
Met (M)	0	0.0%
Phe (F)	0	0.0%
Pro (P)	177	37.5%
Ser (S)	0	0.0%
Thr (T)	118	25.0%
Trp (W)	0	0.0%
Tyr (Y)	0	0.0%
Val (V)	0	0.0%

Supplemental Table 1: Amino acid compositions in the tandem repeats of human PRG4 isoform A and SynLubricin.