

Supplementary Data

A vaccine displaying a trimeric influenza-A HA stem protein on capsid-like particles elicits potent and long-lasting protection in mice

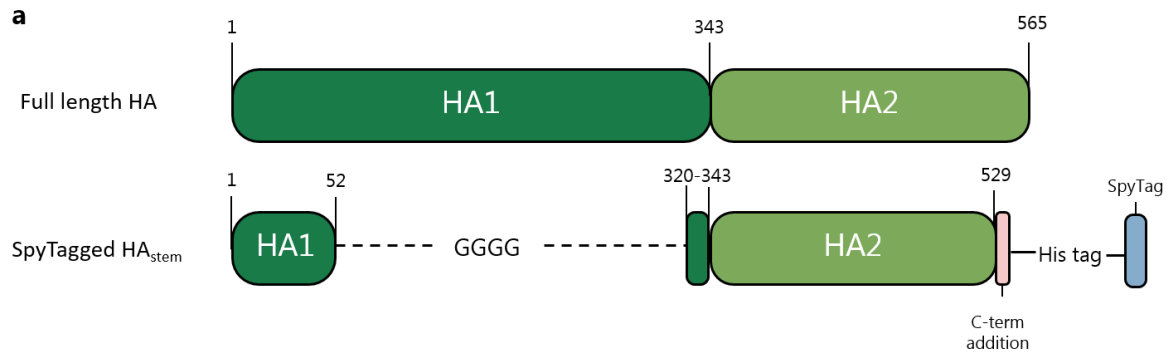
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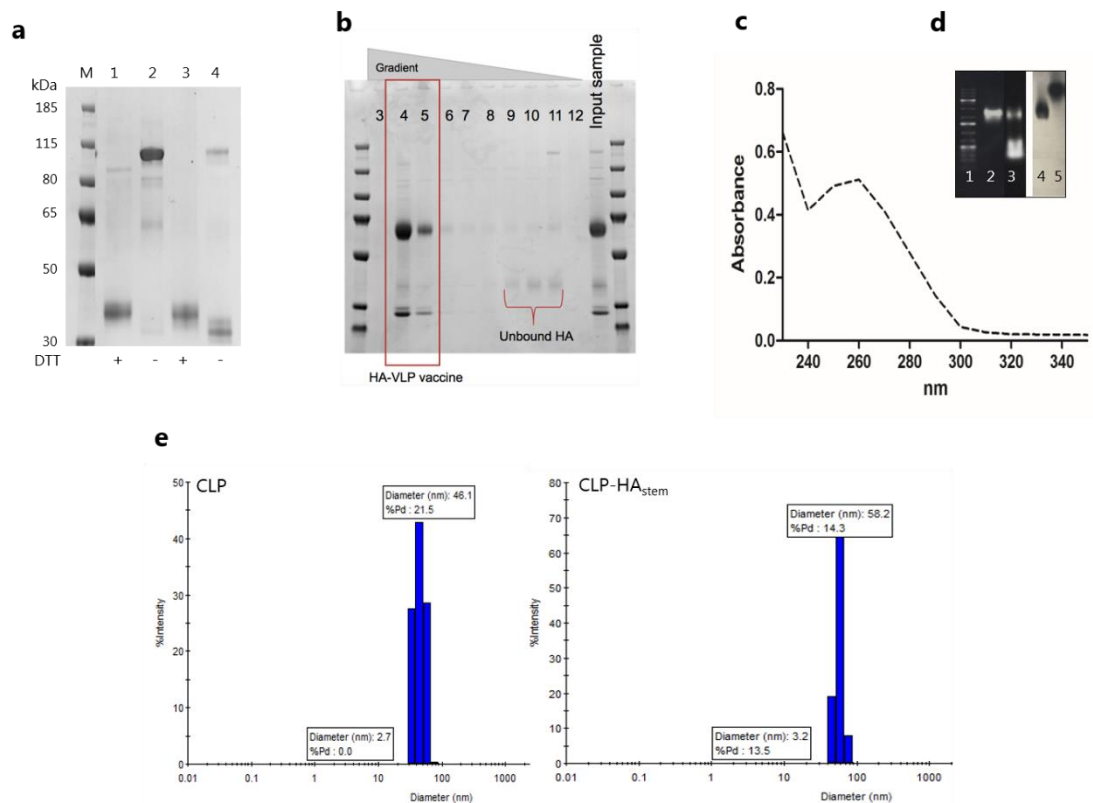
b

	1	21	41
SpyTagged HA _{stem}	MKVLLVLLCTFTATYADTI	CIGYHANNSTDVDTVLEKN	VTVTHSVNLEN
A/Brisbane/59/2007 (H1N1)	MKVLLVLLCTFTATYADTI	CIGYHANNSTDVDTVLEKN	VTVTHSVNLEN
A/Puerto Rico/8/1934 (H1N1)	MKANLLVLLCALAADADI	CIGYHANNSTDVDTVLEKN	VTVTHSVNLEN
	61	81	101
SpyTagged HA _{stem}	SAKLRMTGLRN	PSQS	QSG
A/Brisbane/59/2007 (H1N1)	SAKLRMTGLRN	PSQS	QSG
A/Puerto Rico/8/1934 (H1N1)	SAKLRMTGLRN	PSQS	QSG
	121	141	161
SpyTagged HA _{stem}	QNAINGITNKVNSVIEKMNT	QTAIG	ENK
A/Brisbane/59/2007 (H1N1)	QNAINGITNKVNSVIEKMNT	QTAIG	ENK
A/Puerto Rico/8/1934 (H1N1)	QNAINGITNKVNTVIEKMNI	QTAIG	ENK
	181	201	221
SpyTagged HA _{stem}	LENERTLDFHDSNVKNLYEK	VKSQKNNNAKEIGNGCFEFY	HKCNDECMESVKNGTIDYDPK
A/Brisbane/59/2007 (H1N1)	LENERTLDFHDSNVKNLYEK	VKSQKNNNAKEIGNGCFEFY	HKCNDECMESVKNGTIDYDPK
A/Puerto Rico/8/1934 (H1N1)	LENERTLDFHDSNVKNLYEK	VKSQKNNNAKEIGNGCFEFY	HKCNDECMESVKNGTIDYDPK
	241	261	281
SpyTagged HA _{stem}	YSEESKLNREKIDGVKLESM	GVYQI	EGRHHHHHGGAHIV
A/Brisbane/59/2007 (H1N1)	YSEESKLNREKIDGVKLESM	GVYQI	-----
A/Puerto Rico/8/1934 (H1N1)	YSEESKLNREKIDGVKLESM	GVYQI	-----

Supplementary Fig. 1 Sequence comparison of SpyTagged HA_{stem} with wild type HA proteins

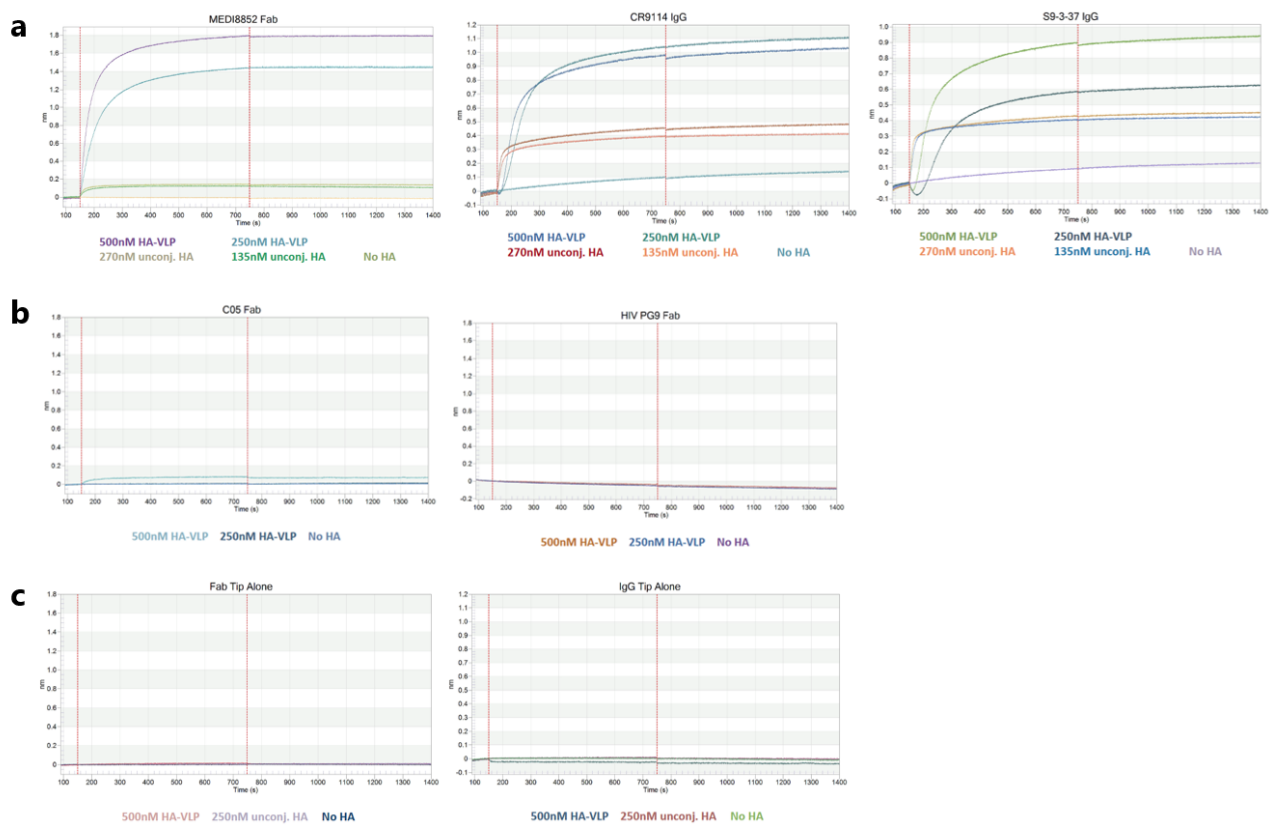
a. Graphical depiction of Spytagged HA_{stem} antigen compared to full length A/Brisbane/59/2007 HA.

b. Sequence alignment of Spytagged HA_{stem} with corresponding amino acid segments from A/Brisbane/59/2007 and A/Puerto Rico/8/1934 H1N1 viral strains. Residues engineered into SpyTagged HA_{stem}, which are not present in the A/Brisbane/59/2007 parental protein, are highlighted in green. Non-conserved amino acid residues between the A/Brisbane and A/PR8 strains are colored red. There is a 93% pairwise identity between the A/Brisbane and A/PR8 HA_{stem} sequences compared to an 86.5% identity between the full-length HA proteins of the two strains.



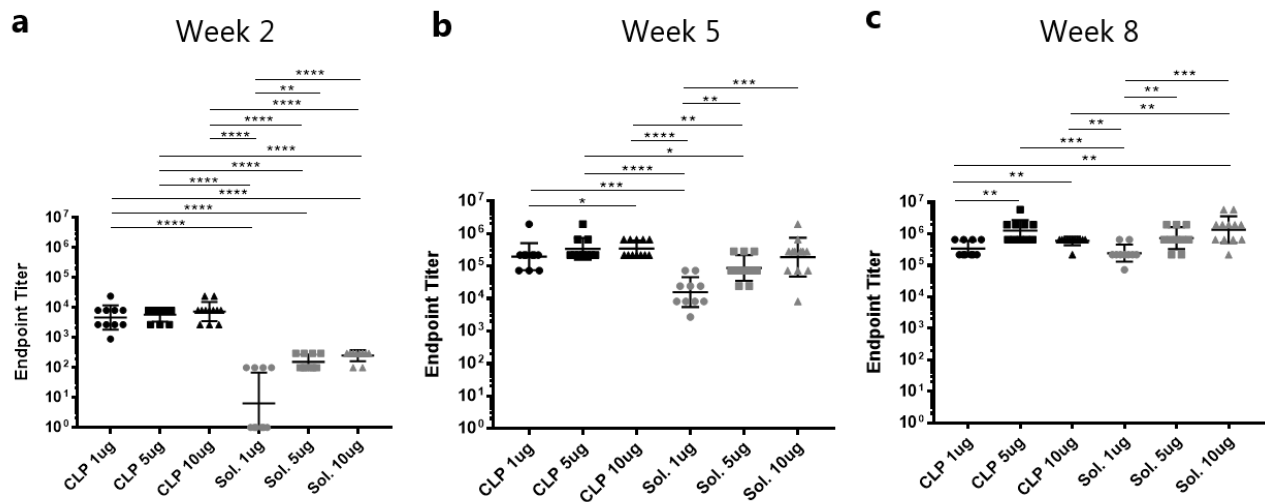
Supplementary Fig. 2 Characterization of purified HA_{stem} and CLP-HA_{stem}.

a Purification of HA_{stem} trimer via immobilized metal affinity chromatography followed by size exclusion chromatography. SDS-PAGE run under reducing (+DTT) and non-reducing (-DTT) conditions, containing a fraction eluted early during gel filtration, and thus containing predominantly trimeric HA_{stem} (lane 1 and 2), and a fraction eluted later on, containing predominantly monomeric HA_{stem} (lane 3 and 4). **b** Purification of CLP-HA_{stem} by density gradient ultracentrifugation. Fraction 3 (high density) to 12 (low density) run on reducing SDS-PAGE alongside input sample taken before ultracentrifugation. Fraction 4 and 5 were pooled and used for the immunization studies. **c** UV spectrum of purified CLP. RNA absorption dominates (260nm). **d** Agarose gel analysis of AP205 CLP. 1kb DNA size marker (lane 1), AP205 CLP input (lane 2 and 4), Urea denatured AP205 CLP (lane 3+5). Left ethidium bromide stained gel, right coomassie stained gel. **e** Dynamic light scattering (DLS) analysis of uncoupled CLP (left) and CLP-HA_{stem} vaccine (right).



Supplementary Fig. 3 Binding of bnAb to HA_{stem} and CLP-HA_{stem}.

Bio-layer interferometry analysis of the binding of **a** MEDI8852 Fab (*left*), CR9114 IgG (*center*) and S9-3-37 IgG (*right*) bnAbs; **b** C05 Fab (HA-head specific) (*right*) and HIV PG9 Fab (HIV specific) (*left*); **c** Fab (*left*) or IgG (*right*) tip alone, to soluble HA_{stem} (270mM and 135nM) and CLP-HA_{stem} (500nM and 250nM). Each plot is one representative of 3 independently performed experiments.



Supplementary Fig. 4 HA_{stem} specific IgG titres from dose escalation study

ELISA measurements of HA_{stem} specific IgG titres from serum taken at **a** Week 2, **b** Week 5 and **c** Week 8. Cut off was set to OD_{450nm} of 0.2. Each dot represents one animal. Horizontal lines indicate geometric mean of the group and vertical lines indicate the standard deviation. *p<0.05; **p<0.005; ***p<0.0005, ****p>0.00005.