Adapting ferritin, a naturally occurring protein cage, to modulate intrinsic agonism of OX40

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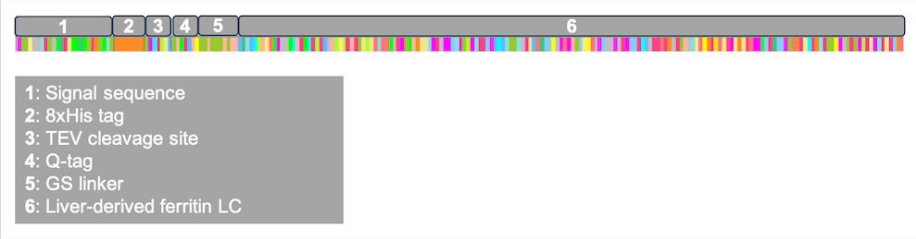

Figure S1. Primary structure of liver-derived ferritin LC, including signal sequence for protein secretion.

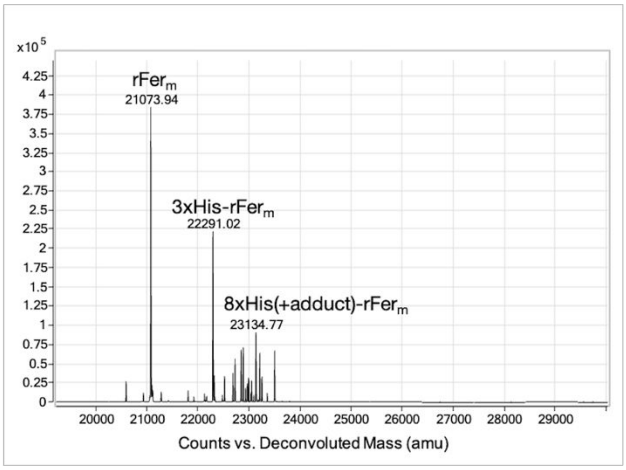

Figure S2. Full deconvoluted spectrum demonstrates that while most of the protein was completely cleaved, there were secondary masses corresponding to a ragged His tag.

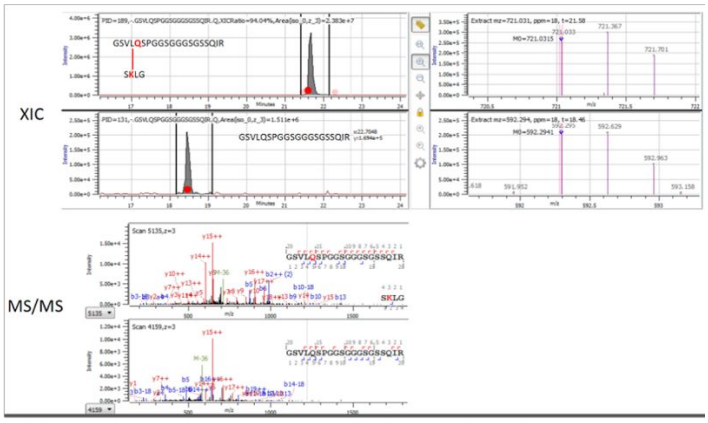

Figure S3. Ion count and MS/MS raw spectra for $rFer_m$-anti-OX40 Fab.

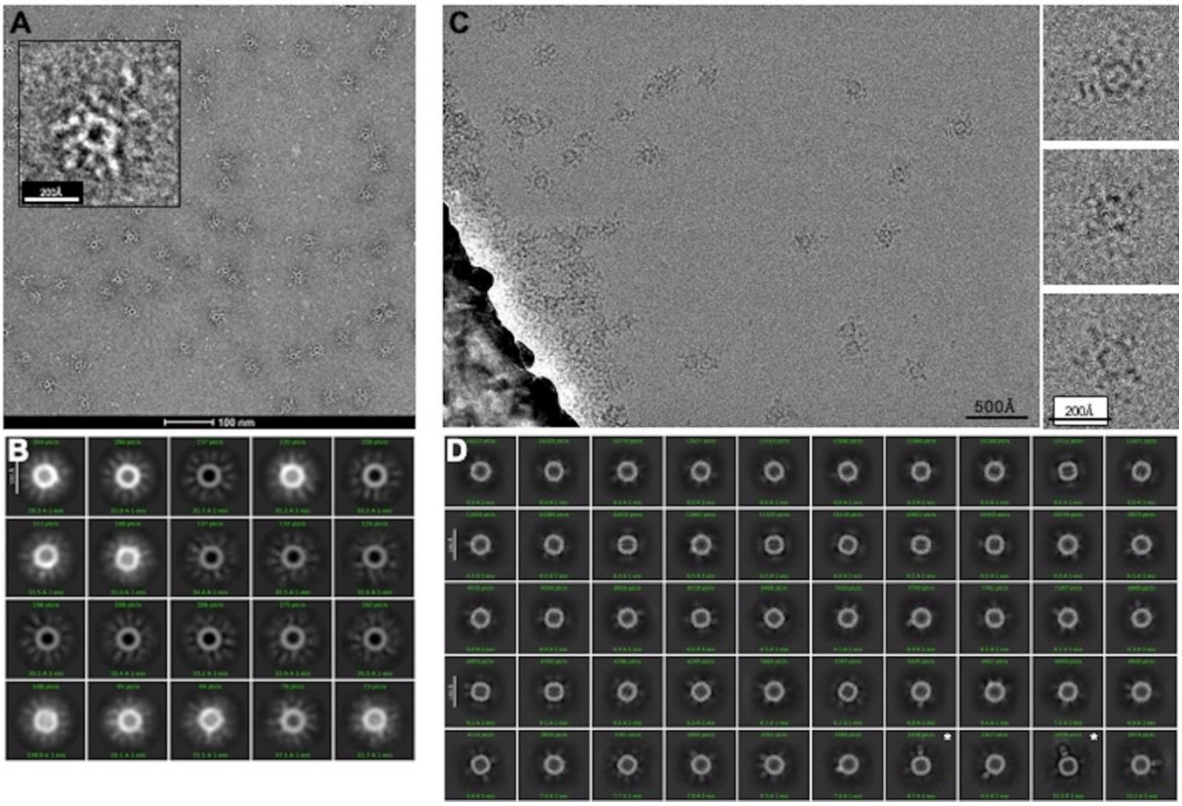

Figure S4A. A negative stain micrograph of the rFe_{r24}-OX40 Fab_{24} conjugate sample. Individual particles clearly show fabs bound to the rFe_{r24} in a variety of poses. A single, magnified raw particle is in the inset. Some Fabs dissociate at this low concentration of protein and can be seen as small particles in the background of the image. **B.** 20 of the 50 2D class averages determined from 3500 particles extracted in a 420Å box. All of these averages show ~12 Fab densities emanating from the rFe_{r24}. **C.** A cryo-EM micrograph of the rFe_{r24}-OX40 Fab_{24} conjugate sample frozen in vitreous ice. Three individual particles are magnified that show fabs bound in drastically different poses. **D.** 50 of the 200 2D class averages determined from 630,000 particles extracted from these images. Helices are clearly visible in the rFe_{r24} cage indicating the high-resolution information contained in these images. The Fabs are mostly average out into blurry densities or averaged out all together because of the variety of orientations their flexible linkers allow them to adopt. In some of the averages with fewer particles (white asterisks), one of the Fabs is able to dominate the alignment enough to resolve at least two views of a characteristic Fab fold.

Table S1. Peptide mapping of isopeptide bond between $rFer_m$ and anti-OX40 Fab

Ret. Time (min)	m/z	Peptide 1	Peptide 2	x-linked AA
21.42	721.0329 [MH+3]	GSVL**Q**SPGGSGGGSGSSQIR ($rFer_m$)	RS**K**LG (Fab)	Q5-K224