Supplementary Information

HiBiT-qIP, HiBiT-based quantitative immunoprecipitation, facilitates the determination of antibody affinity under immunoprecipitation conditions

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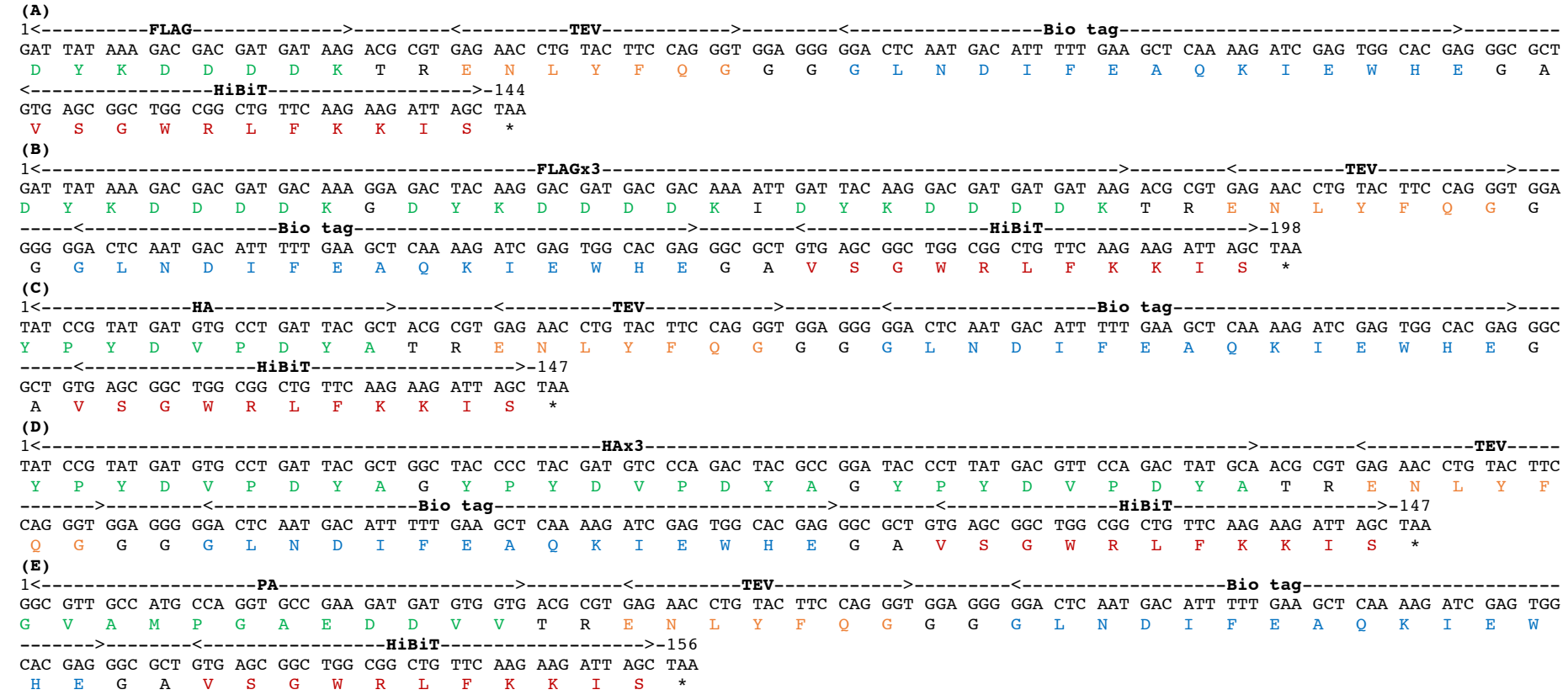
Figure S1: Nucleotide and amino acid sequences of composite epitope tags



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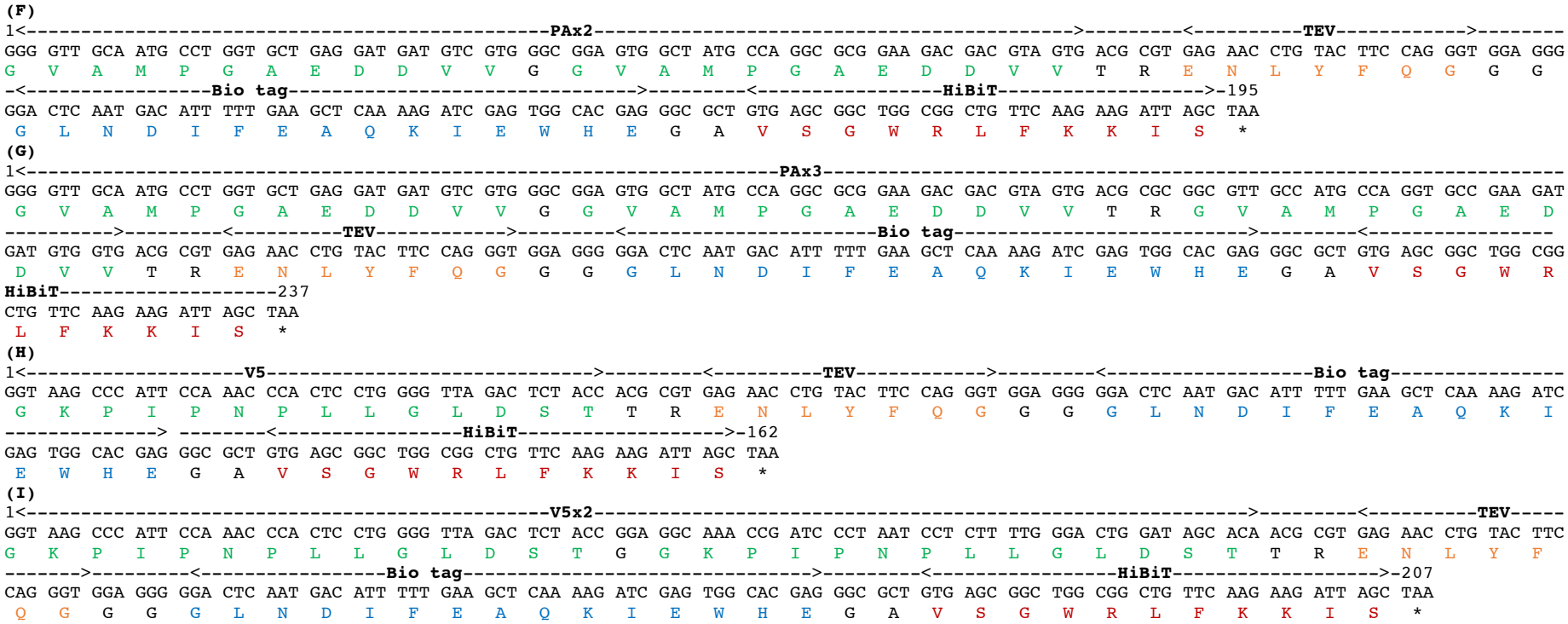

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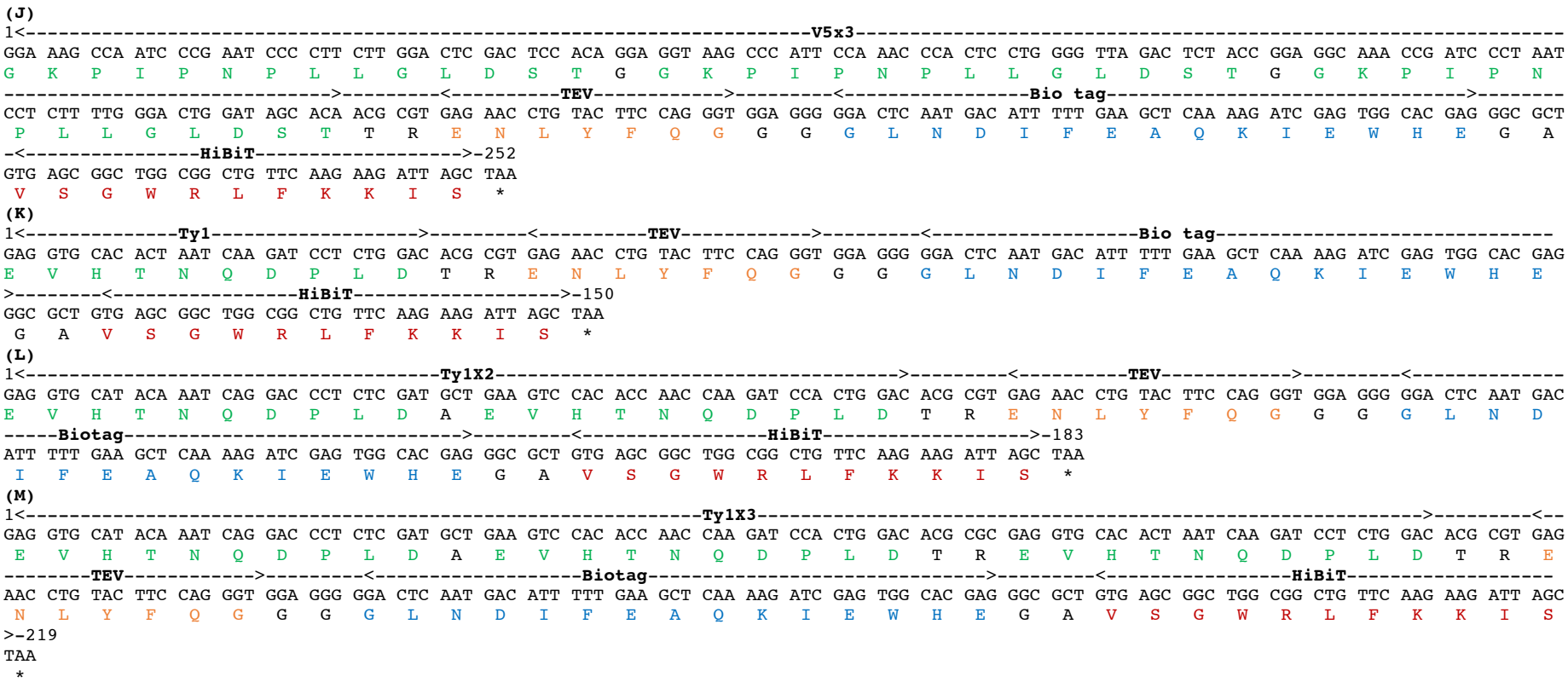
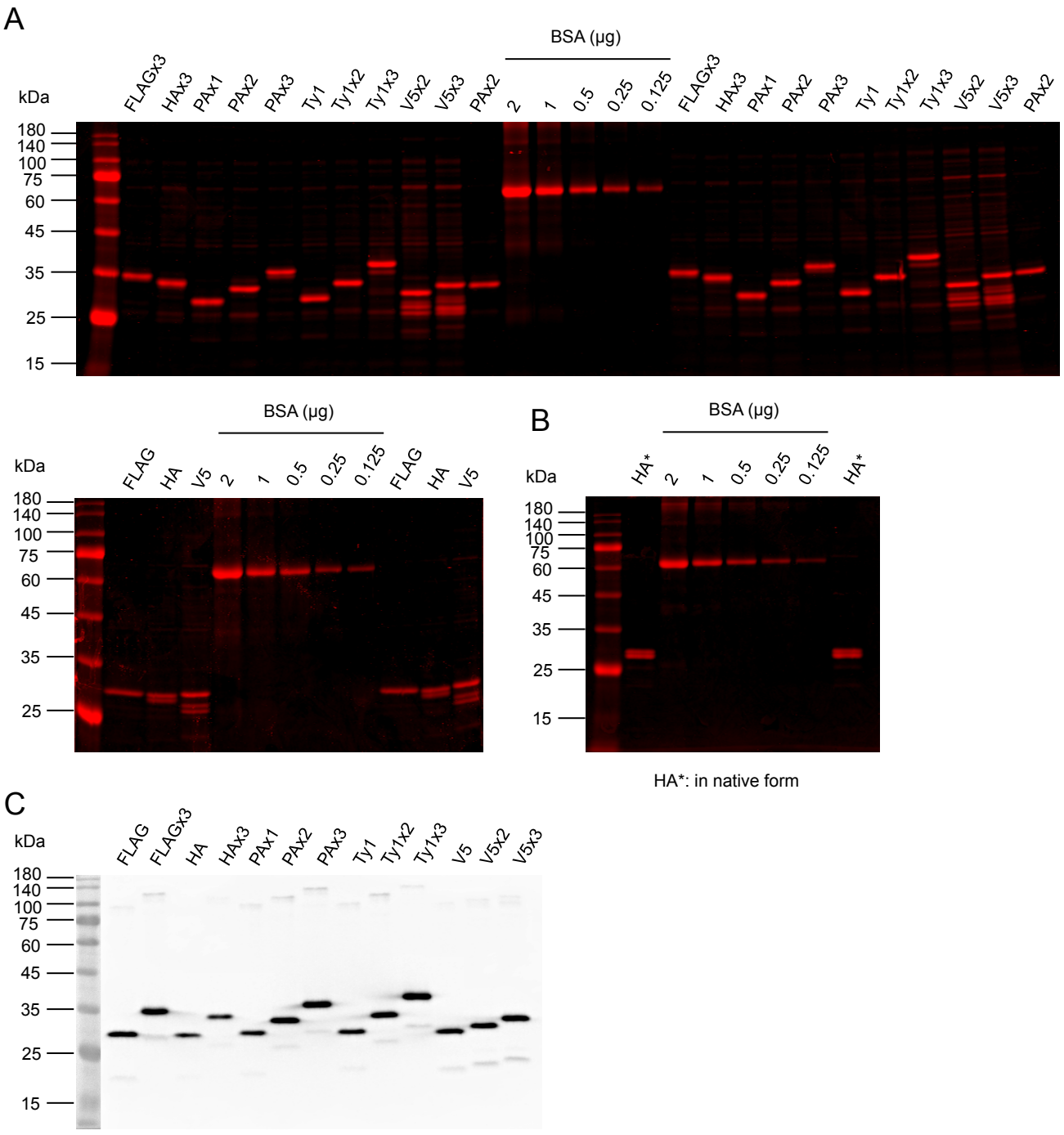
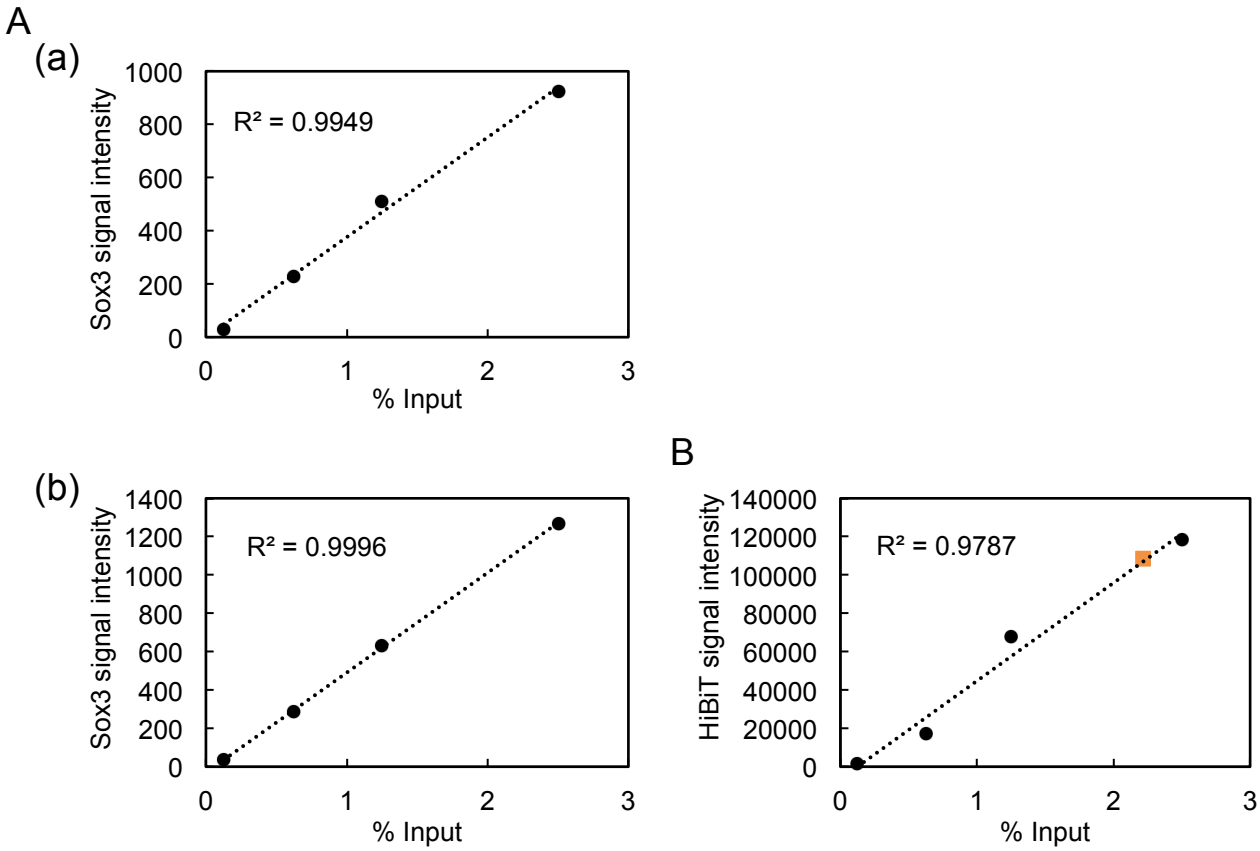


Supplementary Figure 1. Nucleotide and amino acid sequences of the composite epitope tags. The amino acid sequences of the composite epitope tags are shown with the corresponding nucleotide sequences. Each nucleotide sequence is preceded by the *Xho*I site sequence CTCGAG and followed by the *Xba*I-*Not*I linker sequence TCTAGAGCGGCCGC for cloning into pGEX-6P-1. TEV, TEV protease cleavage site; Bio tag, biotin ligase recognition site.



Supplementary Figure 2. Quantification of GST-epitope tag fusion protein. (**A**) SDS polyacrylamide gel images of GST proteins fused with monomeric, dimeric and trimeric forms of the epitope tags, which were prepared in denatured form. Each GST fusion was loaded in duplicate, and BSA standards were loaded for quantification. The gels were stained with Coomassie Brilliant Blue G-250 and detected by near-infrared fluorescence. (**B**) SDS polyacrylamide gel image of the GST protein fused with a monomeric form of the HA epitope tag, which was prepared in native form. (**C**) HiBiT blot detection of the series of tagged GST proteins to confirm retention of the C-terminal HiBiT tag.



Supplementary Figure 3. Standard curves demonstrating the linear correlation of the signal intensity with the percent input. (**A**) The near-infrared fluorescence (NIR) signals were linear across the entire range of percent inputs tested, with $R^2 > 0.99$ for the Sox3 protein tagged with FLAG in monomeric (a) or trimeric (b) form. (**B**) The HiBiT-derived bioluminescence was linear across the entire range of percent inputs tested, with $R^2 > 0.97$ for the FLAGx3-tagged-Sox3 protein. The signal intensity for 0.5 ng of FLAGx3-tagged GST was plotted on the calibration curve (orange square).

Epitope tag antibody	K_d (nM)	Assay format/ method	References
FLAG (M2)	0.76	HiBiT-qIP	This study
	28	SPR	Fujii et al. (2014) [35]
	6.7	SPR	Wegner et al. (2002) [51]
	3	Saturation binding and Scatchard analysis	Firsov et al. (1996)[52]
HA (4B2)	6.6	HiBiT-qIP	This study
	1.6	SPR	Fujii et al. (2014) [35]
PA (NZ-1)	0.65	HiBiT-qIP	This study
	0.40	SPR	Fujii et al. (2014) [35]

Supplementary Table 2. Comparison of the K_d values obtained in this study with those reported in the literature. To the best of our knowledge, all the available K_d values for the interactions of monoclonal antibodies with the epitope tags used in this study are listed in this table.