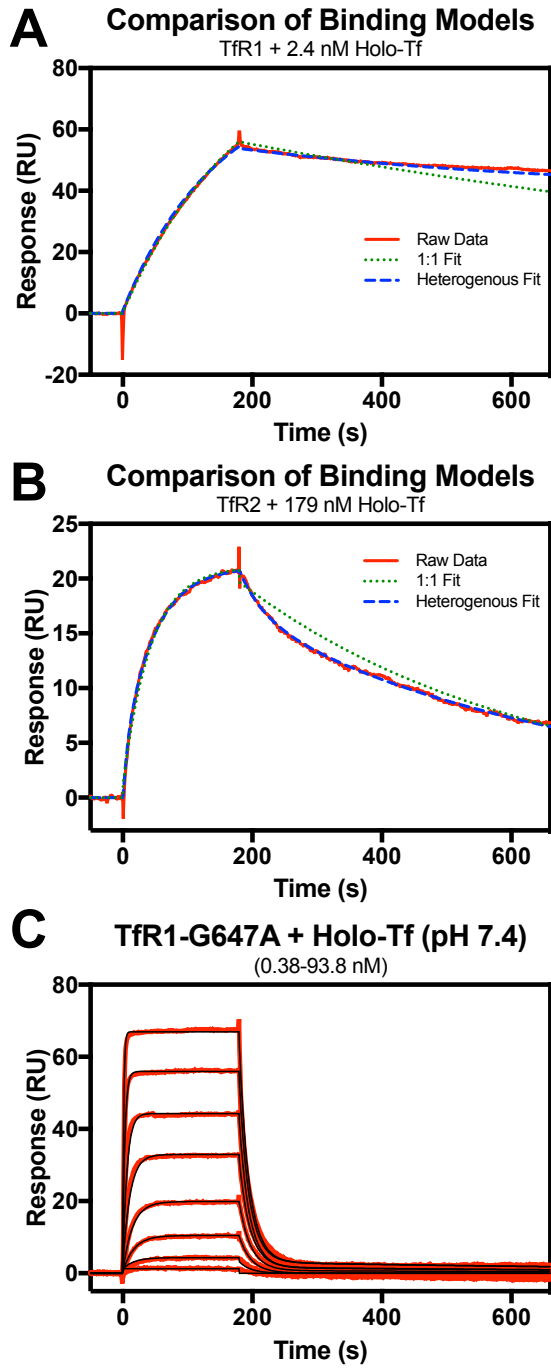
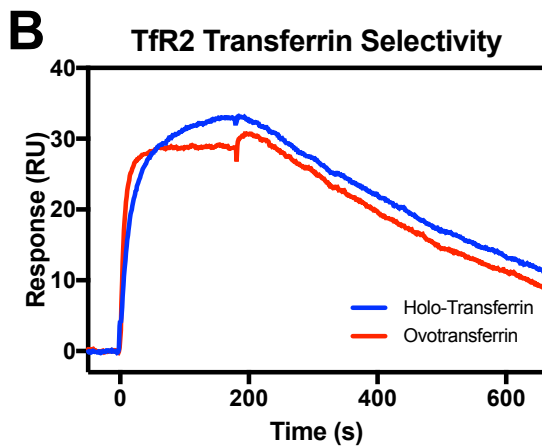
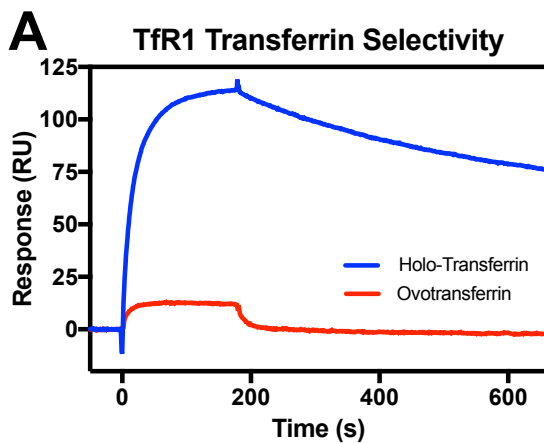


Supplemental Figure 1



Supplemental Figure 1. The Tf-binding behavior of full-length Tf receptor. Comparison of 1:1 and heterogenous binding models applied to raw surface plasmon resonance data. Representative curves from TfR1 (A) and TfR2 (B) are shown. (C) The interaction of holo-Tf with TfR1-G647A mutation by SPR. ~200 RU's of the receptor were immobilized to the chip surface, followed by sequential injections of 0.38 – 93.8 nM of holo-Tf (2.5-fold dilution series). Reference cell-subtracted binding response data is indicated in red, and heterogenous model fits are shown in black. Extrapolated kinetic constants from the model fits are shown in Table 1.

Supplemental Figure 2



Supplemental Figure 2. The analyte specificity of full-length Tf receptor. (A) SPR binding responses of TfR1 to holo-Tf (37.5 nM) or ovo0Tf (960 nM). (B) SPR binding responses of TfR2 to holo-Tf or ovo-Tf (both at 960 nM). ~200 RU's of the receptor were immobilized to the chip surface, followed by injections of Tf solutions.

Supplemental Table 1. Primers used in transferrin receptor cloning and site-directed mutagenesis.

pEGFP-C2 Cloning Primers		
TfR1	Forward	AGTCCGGCCGGACTCAGATCTCGAGAATGATGGATCAAGCTAGATC
	Reverse	ATCCCGGGCCCGCGGTACCGTCGACTTAAAACCTATTGTCAATGTC
TfR2	Forward	GGATACTCGAGACTCGTCCCCCGCGGCTCCGAGCGGCTTTGGGGTC
	Reverse	GGATAGTCGACTCAGAAGTTGTTATCAATGTTCCAGACA
TfR2-TfR1-HD	TfR2(1-636) - Forward	AGTCCGGCCGGACTCAGATCTCGAGAGAGCGGCTTTGGGGTCTATTC
	TfR2(1-636) - Reverse	TCAATTCAACATCGTGGCTGAGCCGGATG
	TfR1(605-760) - Forward	CAGCCACGATGTTGAATTGAACCTGGAC
	TfR1(605-760) - Reverse	ATCCCGGGCCCGCGGTACCGTCGACTTAAAACCTATTGTCAATGTC
Site-Directed Mutagenesis Primers		
TfR1-H318Q	Forward	TCCCTTCCTTCAATCAGACTCAGTTTCCACCA
	Reverse	TGGTGGAAGGAACTGAGTCTGATTGAAGGAAGGGAA
TfR1-E759N	Forward	TGGGACATTGACAATAACTTTAAGTCGACGGT
	Reverse	ACCGTCGACTTAAAAGTTATTGTCAATGTCCCA
TfR2-Q340H	Forward	TCCCTTCCTTCAATCACACCCAGTTCCTCCCA
	Reverse	TGGAGGGAAGGAACTGGGTGTGATTGAAGGAAGGGAA
TfR2-N800E	Forward	TGGAACATTGATAACGAGTTCTGAGTCGACGGTACC
	Reverse	GGTACCGTCGACTCAGAAGTTCGTTATCAATGTTCCA

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