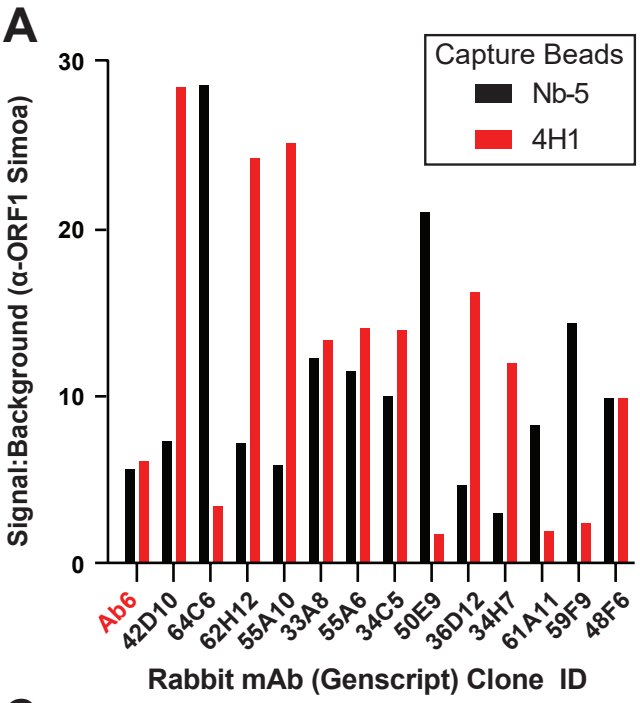

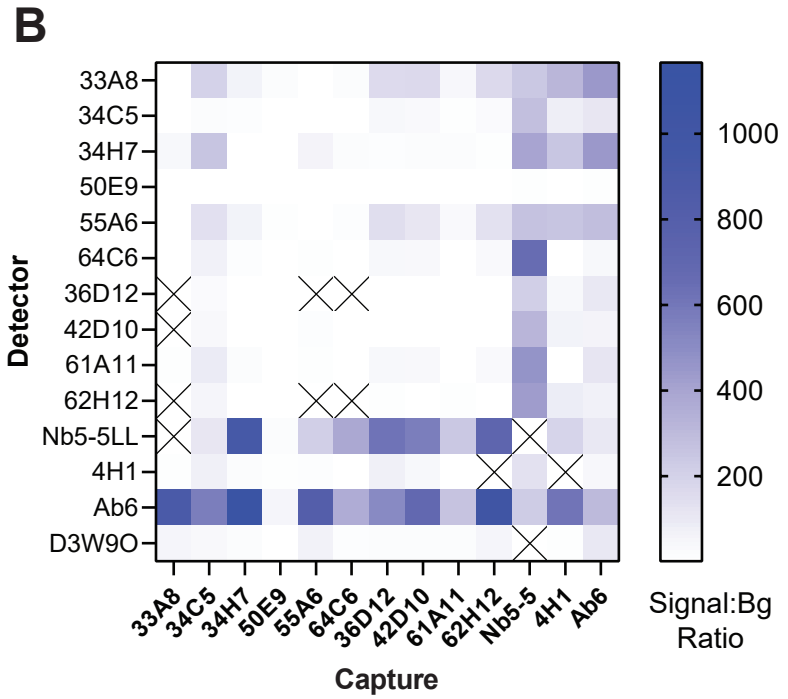

C

		DETECTOR								
		Ab6	**Nb5-5LL**	**33A8**	**34C5**	**34H7**	**64C6**	**61A11**	**62H12**	**55A6**
CAPTURE	**33A8**	0.016								
	34C5	0.026				0.032				
	34H7	0.006	0.043							
	55A6	0.026	0.055							
	64C6	0.034	0.041							
	36D12	0.046	0.050							
	42D10	0.021	0.020							
	61A11	0.042	0.066							
	62H12	0.008	0.062							
	Nb5-5	0.050		0.133	0.022	0.047	0.038	0.042	0.120	
	4H1	0.030	0.056	0.057		0.044				0.036
	Ab6	0.102	0.044	0.063					0.113	

Limit of Detection (pg/mL)

Supplementary Figure 12. Screening of newly developed monoclonal antibody candidates (GenScript). (A) Signal:background ratios of novel rabbit monoclonal α-ORF1p antibodies (B-cell supernatants) in a modified Simoa employing the candidate mAb plus biotinylated secondary anti-Rabbit pAb as detector; these show up to 5-fold improvement vs. our prior best detection antibody, Ab6. Two different capture beads with distinct epitopes were employed. (B-C) Best performers were then synthesized and purified; mAbs were used in further screening with a dimeric nanobody and commercially available monoclonal antibodies for ORF1p detection with Simoa. (B) Signal-to-background comparisons of affinity reagents as capture/detector pairs on Simoa, using recombinant ORF1p protein. All labeled affinity reagents except Nb5-5LL are monoclonal antibodies; Nb5-5LL denotes a homodimeric form of the nanobody Nb5. (C) Limit of detection values for affinity reagent pairs selected from screening in (B). Anti-ORF1 D3W9O is from Cell Signaling Technology.