

1 **Supplementary Table S1. Serological results of heifers immunized with S19-GFP**  
 2

Vaccination protocol	Heifer	Assay	Weeks after vaccination																	
			0	2	4	6	7	8	10	12	16	20	24	28	32	38	42	50	64	130
<b>Group A</b> Subcutaneous S19-GFP	1227	RBT	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
		ELISA-GFP	-	-	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-	-
	73LM	RBT	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	ND
		ELISA-GFP	-	-	-	-	-	-	+	+	+	+	-*	-	-	-	-	-	-	ND
	95A	RBT	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
		ELISA-GFP	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
	424	RBT	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
		ELISA-GFP	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-
	429	RBT	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
		ELISA-GFP	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
<b>Group B</b> Subcutaneous S19-GFP/GFP 7 weeks after GFP boost	1217	RBT	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	
		ELISA-GFP	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
	1218	RBT	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
		ELISA-GFP	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
	1221	RBT	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
		ELISA-GFP	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-
	1223	RBT	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
		ELISA-GFP	-	-	-	-	-	+	+	-	+	-	-*	-	-	-	-	-	-	-
	70LM	RBT	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
		ELISA-GFP	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	72LM	RBT	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
		ELISA-GFP	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
	70A	RBT	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		ELISA-GFP	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
428	RBT	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	
	ELISA-GFP	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	

	431	RBT	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
		ELISA-GFP	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
	435	RBT	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
		ELISA-GFP	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+
<b>Group C</b> Subcutaneous S19-GFP 7 weeks after GFP boost	1219	RBT	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	
		ELISA-GFP	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	1222	RBT	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
		ELISA-GFP	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-
	1230	RBT	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
		ELISA-GFP	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
	76A	RBT	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
		ELISA-GFP	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-
436	RBT	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	
	ELISA-GFP	-	-	-	-	-	+	+	+	+	+	-*	-	-	-	-	-	-	+	
<b>Group D</b> Conjunctival S19-GFP 7 weeks after GFP boost	425	RBT	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		ELISA-GFP	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
	426	RBT	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		ELISA-GFP	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
	432	RBT	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		ELISA-GFP	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
	433	RBT	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		ELISA-GFP	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
434	RBT	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	
	ELISA-GFP	-	-	-	+	+	+	+	+	+	+	-*	-	-	-	-	-	-	-	

3

4 Results are RBT and iELISA-GFP, exception for week 130, which were tested also by WB for GFP. (-) Negative reaction; (+)  
5 Positive reaction. (\*) Indicates positive reaction in WB for GFP, (ND) not determined.

6

7

8