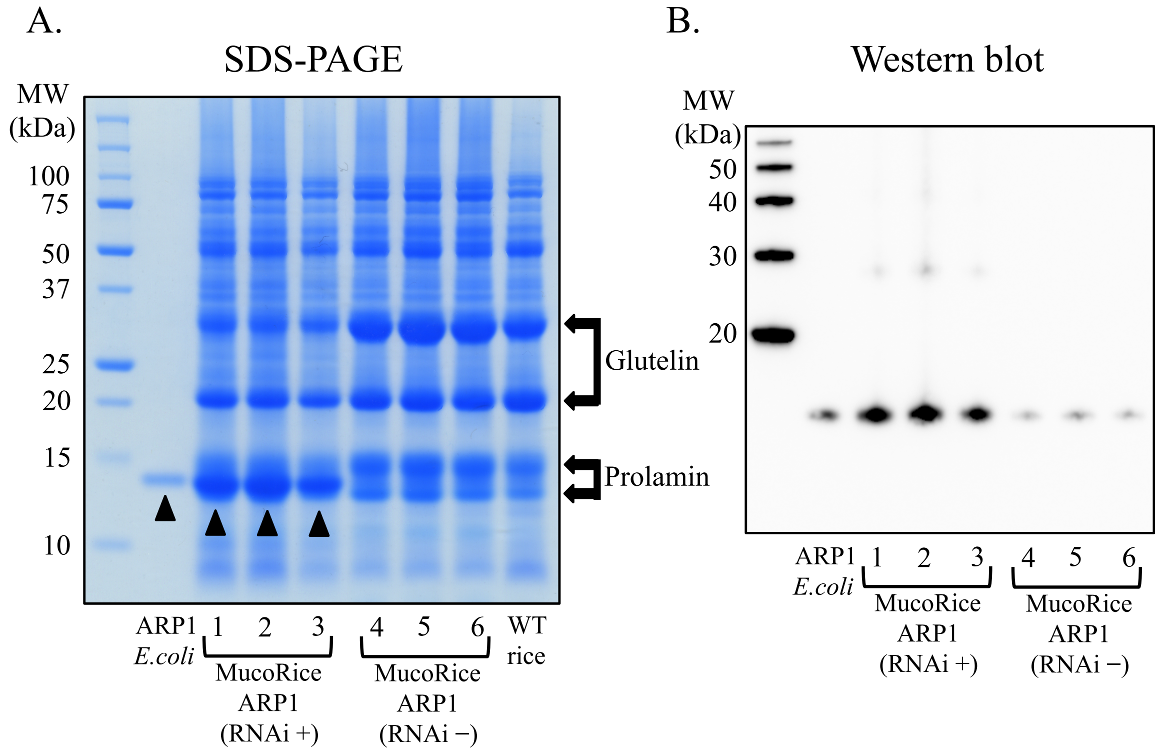
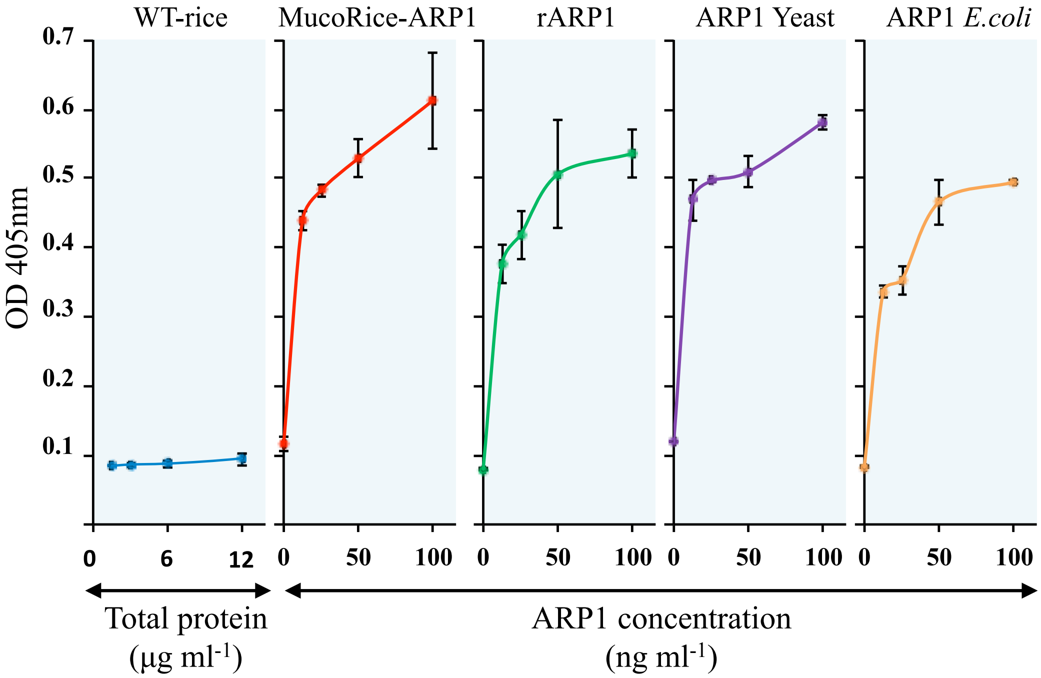
Supplementary Information

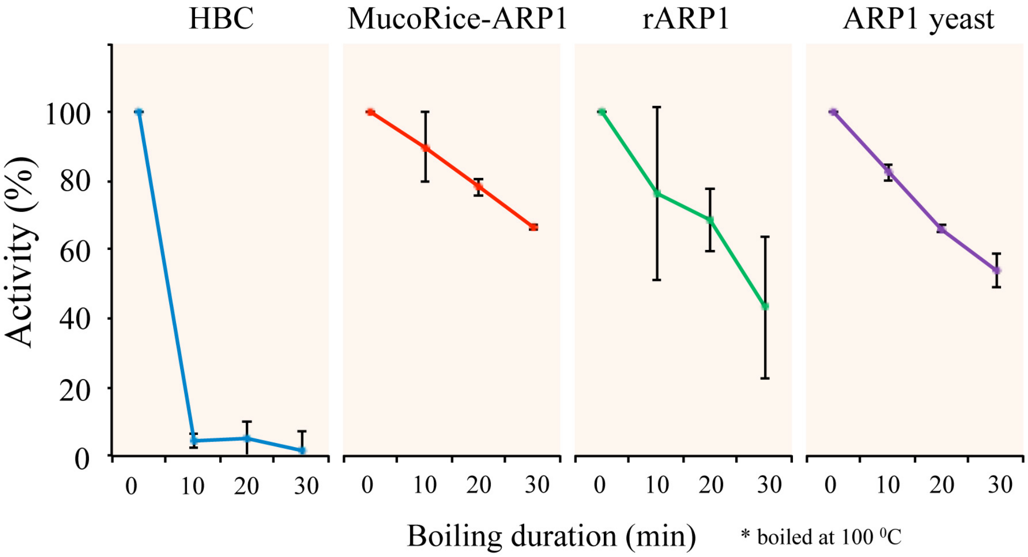
Supplementary Figure 1.



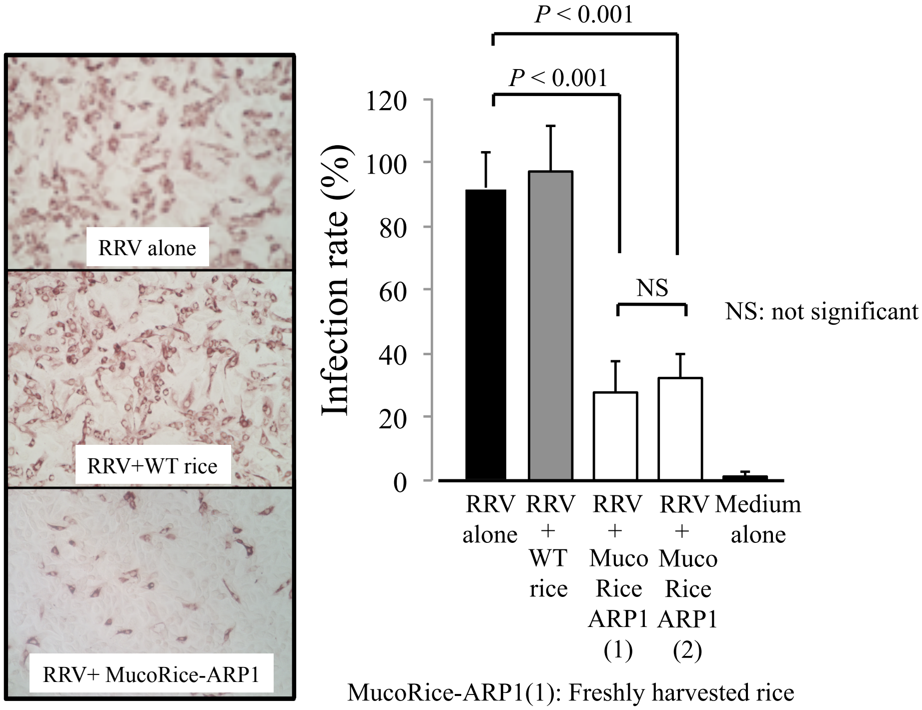
Supplementary Figure 2.



Supplementary Figure 3.



Supplementary Figure 4.

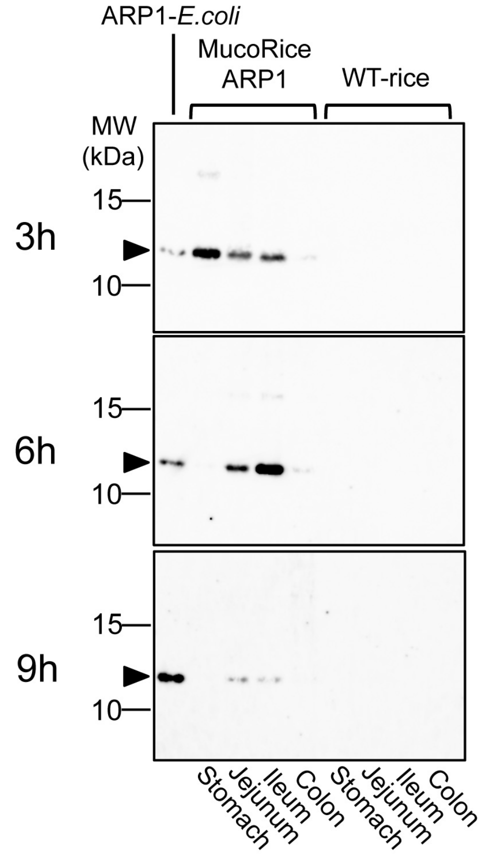


Host cell: MA 104

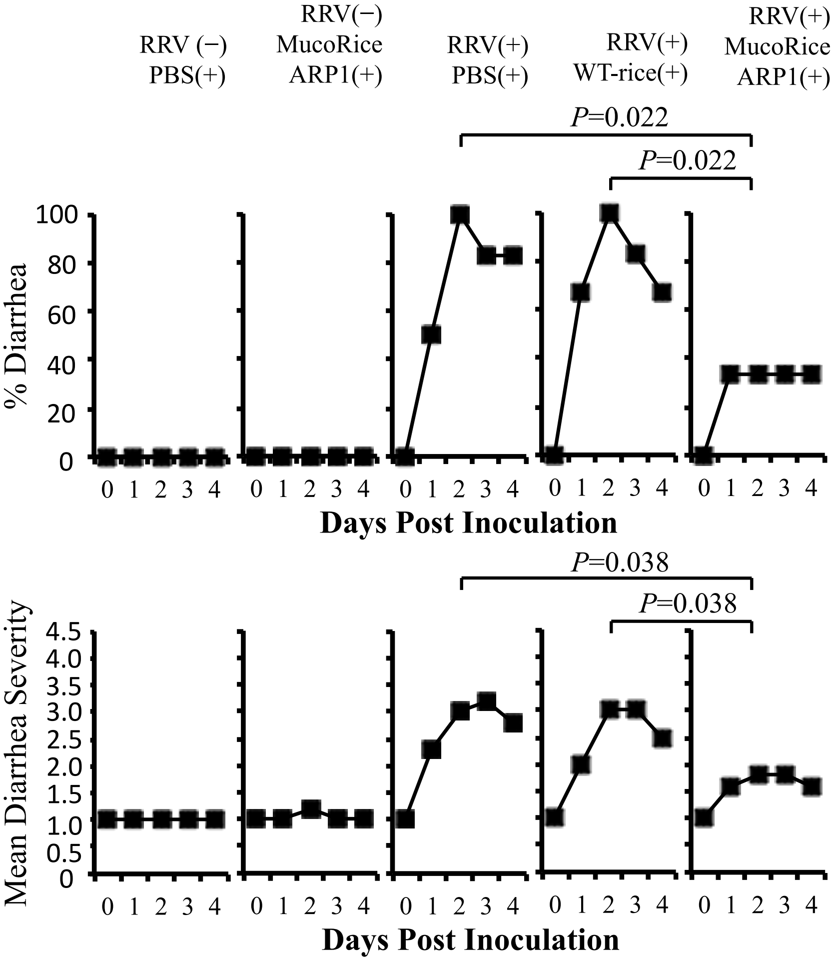
MucoRice-ARP1(1): Freshly harvested rice

MucoRice-ARP1(2): Long-term stored at room temperature (> 1yr)

Supplementary Figure 5.



Supplementary Figure 6.



Supplementary Figure Legends

Supplementary Figure 1. Comparison of ARP1 expression in MucoRice-ARP1 with or without RNAi suppression of rice endogenous storage proteins.

ARP1 expression was compared between MucoRice-ARP1 with RNAi suppression of production (RNAi+) of the major rice endogenous storage proteins, prolamin (13kDa) and glutelin (22 and 33 kDa) (Lane 1-3), and MucoRice-ARP1 without RNAi suppression (RNAi-) (Lane 4-6). Each lane (Lane 1-6) of MucoRice-ARP1 corresponds to a separate rice seed. (**A**) MucoRice-ARP1 (RNAi+) showed a major reduction of glutelin and prolamin (arrows) compared to MucoRice-ARP1 (RNAi−), furthermore, MucoRice-ARP1(RNAi+) showed a high expression of ARP1 (170 μg/seed) with a molecular weight of 12 kDa (arrowheads) compared to MucoRice-ARP1 (RNAi−) (14 μg/seed). (**B**) Western blot analysis confirmed that ARP1 expression is approximately 10 times higher in MucoRice-ARP1 (RNAi+) than in MucoRice-ARP1 (RNAi-).

Supplementary Figure 2. Binding to RRV of MucoRice-ARP1.

Binding to RRV of ARP1 samples from different origins were determined by ELISA. Two fold dilutions of MucoRice-ARP1 containing rice water (MucoRice-ARP1), wild-type rice (negative control) (WT-rice), purified ARP1 from MucoRice-ARP1 (rARP1), ARP1 purified from *E. coli* (ARP1 *E. coli*) and ARP1 purified from yeast (ARP1 yeast). X-axis of MucoRice-ARP1, rARP1, ARP1 yeast and ARP1 *E. coli* represents ARP1 concentration (ng ml^{-1}) whereas the X-axis of WT-rice represents the equivalent concentration of total WT-rice protein (μg ml^{-1}). Error bars represent standard deviations of duplicates.

Supplementary Figure 3. Heat stability of MucoRice-ARP1.

Samples with 100 ng ml^{-1} of ARP1 (MucoRice-ARP1 containing rice water, ARP1 purified from MucoRice-ARP1 and ARP1 purified from yeast) and HBC containing 100 ng ml^{-1} of total protein, were boiled (100° C) for 10, 20, or 30 min. After cooling, two fold dilutions of each sample were tested by ELISA (see Methods section). The percentage of binding activity was calculated in relation to non-boiled samples tested in the same ELISA plate at a given concentration before reaching binding saturation (25 ng ml^{-1} ARP1 for ARP1 containing samples and 50 ng ml^{-1} total protein for HBC samples). MucoRice-ARP1, MucoRice-ARP1 containing rice water; rARP1, ARP1 purified from MucoRice-ARP1; ARP1 yeast, ARP1 purified from yeast; HBC, protein preparation from hyper-immune bovine colostrum. The mean of two independent experiments is presented. Error bars represent standard deviations of the means.

Supplementary Figure 4. Long-term room temperature stability of MucoRice-ARP1.

Neutralizing activity of freshly harvested MucoRice-ARP1 (1) and long-term stored MucoRice-ARP1 (>1 year at room temperature) (2). MucoRice-ARP1 (1), freshly harvested MucoRice-ARP1; MucoRice-ARP1 (2), long-term stored MucoRice-ARP1 (>1 year at room temperature). To perform the neutralization assays, MucoRice-ARP1 containing rice water (2.1 μg of ARP1) was incubated with 200 ffu of trypsin-activated RRV. The mixtures of MucoRice-ARP1containing rice water and virus were subsequently used to infect MA104 cells. WT-rice and PBS were used as positive controls of infection. As a negative control, MA 104 cells, not infected by RRV, were used. Error bars represent standard deviations of the means.

Supplementary Figure 5. Stability and time course changes of MucoRice-ARP1 localization after intragastric administration.

MucoRice-ARP1 containing rice water (8.5 µg of ARP1) or WT-rice water was intragastrically administered. The presence of rice derived ARP1 in the gut was investigated at 3, 6 and 9 h after administration (n=5, respectively) by Western blot analysis using a polyclonal rabbit anti-ARP1 antibody. ARP1 (arrow) was detected in the entire small intestinal tract of the MucoRice-ARP-treated pups (4 days after birth) at least for 6 hours. On the contrary, ARP1 was not detected in WT-rice-treated pups. In 2 out of 5 MucoRice-ARP-treated mice, ARP1 was detected even 9 h after administration.

Supplementary Figure 6. MucoRice-ARP1 reduced diarrhea in a SCID mouse pup model of rotavirus infection

None of the 4-day-old mice receiving PBS or MucoRice-ARP1 only (RRV-) developed diarrhea while all pups infected with RRV (RRV+) and given PBS or WT-rice developed diarrhea. Neonatal SCID mice receiving MucoRice-ARP1 containing 8.5 µg of ARP1 9 hours after RRV inoculation and subsequently twice daily for 4 consecutive days, had a significantly reduced percent of animals with diarrhea (*P*=0.022) and disease severity (*P*=0.038) compared to pups infected with RRV and administered PBS or WT-rice.

Supplemental table 1. Mass spectrometry of MucoRice-ARP1 by Q-STAR

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M),Acetyl (Protein N-term)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: **63%**
Matched peptides are shown in Bold and underlined below.
SRQVQLQDSGGGLVQAGDRLSLSCAASGRTFSSYDMAWFRQAPGKEREFVAAITTSEGTWYGDAGKGRFTIAR**VNAKNTVYLHMNR**LKPEDTAVYYCAASNQGGSLQISTNYNYWGQGTQVTVSS

Start-End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence	Modification	Ions score
1-19	657.6159	1969.8259	1969.9930	-85	1	SRQVQLQDSGGGLVQAGDR		94
1-19	671.6211	2011.8414	2012.0035	-81	1	SRQVQLQDSGGGLVQAGDR	Acetyl (Protein N-term)	103
1-19	1006.9309	2011.8473	2012.0035	-78	1	SRQVQLQDSGGGLVQAGDR	Acetyl (Protein N-term)	83
1-29	744.1000	2972.3709	2972.4846	-38	2	SRQVQLQDSGGGLVQAGDRLSLSCAASGR		78
3-19	864.3672	1726.7198	1726.8598	-81	0	QVQLQDSGGGLVQAGDR		135
3-19	864.3728	1726.7310	1726.8598	-75	0	QVQLQDSGGGLVQAGDR		127
3-19	864.3755	1726.7365	1726.8598	-71	0	QVQLQDSGGGLVQAGDR		132
3-29	910.7227	2729.1464	2729.3515	-75	1	QVQLQDSGGGLVQAGDRLSLSCAASGR		73
20-29	511.2027	1020.3909	1020.5022	-109	0	LSLSCAASGR		77
20-29	511.2070	1020.3995	1020.5022	-101	0	LSLSCAASGR		80
20-29	511.2177	1020.4209	1020.5022	-80	0	LSLSCAASGR		71
30-40	705.7420	1409.4694	1409.6074	-98	0	TFSSYDMAWFR		79
30-40	705.7571	1409.4996	1409.6074	-77	0	TFSSYDMAWFR		79
30-40	713.7452	1425.4759	1425.6023	-89	0	TFSSYDMAWFR	Oxidation (M)	80
41-66	693.0401	2768.1312	2768.3406	-76	2	QAPGKEREFVAAITTSEGTWYGDAGK		36
48-66	1001.7821	2001.5497	2001.9320	-191	0	EFVAAITTSEGTWYGDAGK		119
48-66	1001.7851	2001.5557	2001.9320	-188	0	EFVAAITTSEGTWYGDAGK		122
48-66	1001.8930	2001.7714	2001.9320	-80	0	EFVAAITTSEGTWYGDAGK		110
74-86	525.8996	1574.6771	1574.7987	-77	1	VNAKNTVYLHMNR	Oxidation (M)	44
78-86	574.2196	1146.4246	1146.5604	-118	0	NTVYLHMNR		50
78-86	574.2400	1146.4654	1146.5604	-83	0	NTVYLHMNR		57

78-86	574.2423	1146.4699	1146.5604	-79	0	NTVYLHMNR		57

Supplemental table 2. Mass spectrometry of MucoRice-ARP1 by Orbitrap

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M),Acetyl (Protein N-term)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: **92%**
Matched peptides are shown in Bold and underlined below.
SRQVQLQDSGGGLVQAGDRLSLSCAASGRTFSSYDMAWFRQAPGK**EREFVAAITTSEGTWYGDAGKGRFTIAR**VNAK**NTVYLHMNRLKPEDTAVYYCAASNQGGSLQISTNYNYWGQGTQVTVSS**

Start-End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence	Modification	Ions score
1-19	657.6714	1969.9923	1969.9930	-0	1	SRQVQLQDSGGGLVQAGDR		75
1-19	657.6715	1969.9927	1969.9930	-0	1	SRQVQLQDSGGGLVQAGDR		70
1-19	986.0039	1969.9933	1969.9930	0	1	SRQVQLQDSGGGLVQAGDR		88
3-19	864.4372	1726.8598	1726.8598	-0	0	QVQLQDSGGGLVQAGDR		129
3-19	864.4377	1726.8608	1726.8598	1	0	QVQLQDSGGGLVQAGDR		127
3-19	864.4377	1726.8609	1726.8598	1	0	QVQLQDSGGGLVQAGDR		130
3-29	910.7912	2729.3518	2729.3515	0	1	QVQLQDSGGGLVQAGDRLSLSCAASGR		55
20-29	511.2582	1020.5019	1020.5022	-0	0	LSLSCAASGR		92
20-29	511.2583	1020.5020	1020.5022	-0	0	LSLSCAASGR		92
20-29	511.2583	1020.5020	1020.5022	-0	0	LSLSCAASGR		92
30-40	705.8108	1409.6071	1409.6074	-0	0	TFSSYDMAWFR		74
30-40	705.8109	1409.6073	1409.6074	-0	0	TFSSYDMAWFR		80
30-40	705.8113	1409.6081	1409.6074	1	0	TFSSYDMAWFR		80
46-66	763.3656	2287.0750	2287.0757	-0	1	EREFVAAITTSEGTWYGDAGK		84
46-66	763.3662	2287.0768	2287.0757	0	1	EREFVAAITTSEGTWYGDAGK		78
46-66	763.3665	2287.0777	2287.0757	1	1	EREFVAAITTSEGTWYGDAGK		87
48-66	1001.9736	2001.9326	2001.9320	0	0	EFVAAITTSEGTWYGDAGK		97
48-66	1001.9740	2001.9334	2001.9320	1	0	EFVAAITTSEGTWYGDAGK		95

48-66	668.3186	2001.9340	2001.9320	1	0	EFVAAITTSEGTWYGDAGK		95
48-68	1108.5334	2215.0523	2215.0546	-1	1	EFVAAITTSEGTWYGDAGKGR		63
67-73	410.7432	819.4718	819.4715	0	1	GRFTIAR		36
78-86	574.2871	1146.5597	1146.5604	-1	0	NTVYLHMNR		57
78-86	574.2872	1146.5599	1146.5604	-0	0	NTVYLHMNR		53
78-86	574.2880	1146.5614	1146.5604	1	0	NTVYLHMNR		53
87-125	1419.9955	4256.9646	4256.9597	1	0	LKPEDTAVYYCAASNQGGSLQISTNYNYWGQGTQVTVSS		41
87-125	1419.9956	4256.9650	4256.9597	1	0	LKPEDTAVYYCAASNQGGSLQISTNYNYWGQGTQVTVSS		33
87-125	1419.9960	4256.9661	4256.9597	2	0	LKPEDTAVYYCAASNQGGSLQISTNYNYWGQGTQVTVSS		35