

## Additional file 3: Raw data from the preliminary immunoreactivity studies

The newly established monoclonal antibodies (mAbs) were tested using ELISAs that targeted the recombinant proteins based on the ectodomain (rHA) or HA1 subunit (rHA1) of the H5 hemagglutinins and the avian influenza viruses (AIVs) of H1-H16 subtypes as antigens (Additional file 1). The same concentrations of individual antibody clones were used for testing. The tests were performed as described in the Methods. The resulting characteristics of the obtained antibody clones are described in the manuscript and summarized in Table 1. The underlying raw data are included in Tables S8, S9 and S10.

**Table S8. The ELISA absorbance values for the selected mAbs tested against recombinant hemagglutinin antigens.**

Antigen name	Hybridoma clones						
	G-1-31-22	G-2-14-10	G-5-32-5	G-6-42-42	G-6-42-71	G-7-24-17	G-7-27-18
<b>Ectodomain-based HA proteins (rHA) from mammalian expression system, conformational</b>							
rHA - A/H5N1/Qinghai	2.440	3.749	3.786	> 4	> 4	2.281	> 4
rHA - A/H5N1/India	2.865	4.000	4.000	> 4	> 4	2.579	> 4
rHA - A/H5N1/Vietnam	2.789	3.922	4.000	> 4	> 4	2.474	> 4
rHA - A/H5N1/Guiyang	3.003	> 4	> 4	> 4	> 4	2.746	> 4
rHA - A/H5N2/California	1.726	3.183	3.566	3.666	3.125	1.514	4.000
<b>Ectodomain-based HA protein (rHA) from mammalian expression system, non-conformational</b>							
rHA - A/H5N1/Ck/Vietnam	0.000	0.004	0.004	0.005	0.006	0.002	0.009
<b>Ectodomain-based HA protein (rHA) from baculovirus expression system, conformational</b>							
rHA - A/H5N1/Poland	1.437	2.692	3.221	3.339	2.663	1.613	3.475
<b>HA1 subunit-based HA proteins (rHA1) from mammalian expression system, conformational</b>							
rHA1 - A/H5N1/Vietnam	2.024	3.427	3.724	> 4	3.557	1.940	> 4
rHA1 - A/H5N1/HK/156	2.334	3.588	3.613	3.784	3.268	2.110	4.000
rHA1 - A/H5N1/HK/483	1.588	2.979	3.262	3.508	2.755	1.420	3.742

The affinity-purified mAbs were analyzed at 0.05  $\mu\text{g}/\text{mL}$  in 2% BSA/PBS on Ni-NTA strips (Qiagen) coated with the recombinant H5 hemagglutinin proteins (1  $\mu\text{g}/\text{mL}$  in 1% BSA/PBS). To control for non-specific binding, the mAbs were also analyzed in the non-coated wells. Commercial antibodies against H5 HA (mAb 8 in Additional file 1: Table S1) were used as a positive control. The blank control was the dilution buffer. The mean absorbance values for blank control samples were subtracted.

**Table S9. The ELISA absorbance values for the selected mAbs tested against AIVs of H1-H16 subtypes.**

Hemagglutinin	Avian influenza virus		Hybridoma clones						
	Subtype	Strain	G-1-31-22	G-2-14-10	G-5-32-5	G-6-42-42	G-6-42-71	G-7-24-17	G-7-27-18
H1	H1N1	A/duck/It/1447/05(H1N1)	0.033	0.014	0.000	0.000	0.000	0.019	0.053
H2	H2N3	A/duck/Germ/1215/73(H2N3)	0.042	0.084	0.005	0.000	0.003	0.032	0.112
H3	H3N8	A/pass/It/6000/V00(H3N8)	0.016	0.051	0.000	0.000	0.000	0.010	0.074
		A/psitt/It/2873/00(H3N8)	0.024	0.018	0.000	0.000	0.000	0.017	0.052
H4	H4N8	A/cockatoo/Eng/72(H4N8)	0.022	0.029	0.002	0.000	0.000	0.011	0.053
H5	H5N1	A/mallard/It/3401/05(H5N1)	0.932	0.826	0.457	1.145	1.126	0.548	1.037
		A/turk/It/80(H5N2)	0.368	0.360	0.229	0.419	0.404	0.204	0.395
		A/duck/It/775/04(H5N3)	1.312	1.474	1.218	1.524	1.717	1.070	1.650
		A/ck/It/22A/98(H5N9)	0.440	0.453	0.326	0.160	0.158	0.356	0.483
H6	H6N2	A/turkey/Canada/65 (H6N2)	0.038	0.039	0.002	0.000	0.002	0.022	0.074
H7	H7N1	A/ck/It/1067/V99(H7N1)	0.072	0.024	0.000	0.000	0.000	0.027	0.062
		A/ty/It/9289/V02(H7N3)	0.043	0.012	0.002	0.001	0.004	0.023	0.055
		A/mallard/It/4810-79/04(H7N4)	0.059	0.030	0.005	0.001	0.003	0.032	0.087
		A/macaw/626/80(H7N7)	0.035	0.038	0.003	0.000	0.000	0.026	0.078
H8	H8N4	A/turk/Ont/6118/68(H8N4)	0.034	0.036	0.001	0.000	0.001	0.030	0.063
H9	H9N2	A/ty/Wis/66(H9N2)	0.020	0.030	0.000	0.000	0.000	0.010	0.048
		A/turk/Scotland/1/70(H9N7)	0.010	0.023	0.000	0.000	0.000	0.006	0.031
H10	H10N1	A/ostrich/SA/01(H10N1)	0.016	0.003	0.000	0.000	0.003	0.014	0.028
H11	H11N6	A/duck/Eng/56(H11N6)	0.017	0.018	0.000	0.000	0.000	0.010	0.036
		A/duck/Memphis/546/174(H11N9)	0.014	0.022	0.001	0.000	0.001	0.013	0.033
H12	H12N5	A/duck/Alberta/60/76(H12N5)	0.025	0.040	0.004	0.000	0.003	0.021	0.055
H13	H13N6	A/gull/Maryland/704/77(H13N6)	0.015	0.028	0.000	0.000	0.000	0.009	0.051
H14	H14N5	A/mallard/Gurjev/263/82(H14N5)	0.015	0.031	0.003	0.000	0.000	0.023	0.029
H15	H15N9	A/shearwater/2576/79(H15N9)	0.026	0.037	0.006	0.000	0.000	0.021	0.041
H16	H16N3	A/gull/Denmark/68110/02(H16N3)	0.053	0.059	0.007	0.000	0.000	0.028	0.095

The affinity-purified mAbs were analyzed at 20 µg/mL in 2% BSA/PBS on MaxiSorp plates (Nunc) coated with the avian influenza viruses (4000 hemagglutination units/mL in PBS). To control for non-specific binding, the mAbs were also analyzed in the non-coated wells. Testing the mAbs with non-H5 subtype AIVs was performed using in parallel the H5N3 and H5N9 viruses to provide additional controls. Commercial antibodies against H5 HA (mAb 8 in Additional file 1: Table S1) were used to serve as a positive control in the assays with H5-subtype AIVs and as a negative control in the assays with AIVs of the H1-H4 and H6-H16 subtypes. The blank control was the dilution buffer. The mean absorbance values for blank control samples were subtracted. The tests were performed in volumes of 50 µL *per well*.

**Table S10. The ELISA absorbance values for the selected mAbs tested against AIVs of the H5 subtype.**

Hemagglutinin	Avian influenza virus		Hybridoma clones						
			G-1-31-22	G-2-14-10	G-5-32-5	G-6-42-42	G-6-42-71	G-7-24-17	G-7-27-18
Subtype	Subtype	strain							
<b>H5</b>	H5N1	A/mallard/It/3401/05(H5N1)	1.166	1.162	0.623	1.604	1.624	0.782	1.573
	H5N2	A/turk/It/80(H5N2)	0.516	0.511	0.435	0.897	0.884	0.350	0.619
	H5N3	A/duck/It/775/04(H5N3)	1.460	1.913	1.381	2.966	3.036	1.438	2.345
	H5N9	A/ck/It/22A/98(H5N9)	0.549	0.651	0.489	0.315	0.291	0.560	0.723

The affinity-purified mAbs were analyzed at 20 µg/mL in 2% BSA/PBS on MaxiSorp plates (Nunc) coated with the avian influenza viruses (4000 hemagglutination units/mL in PBS). To control for non-specific binding, the mAbs were also analyzed in the non-coated wells. Commercial antibodies against H5 HA (mAb 8 in Additional file 1: Table S1) were used as a positive control. The blank control was the dilution buffer. The mean absorbance values for blank control samples were subtracted. The tests were performed in volumes of 100 µL *per* well.