

- 1 **Table S1.** Analysis of virulence determinants in the viral PB2, PB1-F2, PB1, PA, M2, NS1,
 2 and NS2 proteins in the currently 10 Egyptian H9N2 viruses isolated from poultry.

Protein	Site	Virulent	A virulent	Egyptian H9N2 (2013-2015)
PB2	627	K	E	V(8), E (2)
	147	L	M	I
	250	G	V	V
	504	V	I	V
	701	N	D	D
	404	L	F	F
	591	K	Q	K(2), Q(8)
PB1	317	I	M/V	I(8),M(2)
PB1-f2	66	S	N	S(7), N(3)
PA	127	V	I	V
	672	L	F	L
	550	L	I	L
M2	64	S/A/F	P	S
	69	P	L	P
NS1	42	S	A/P	S
	92	E	D	D
	103	L	F	F
	106	I	M	M
	189	N	D/G	D
NS2	31	I	M	M
	56	Y	H/L	H

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7 **Table S2.** Analysis of host range genetic determinants in the PB2, PB1-F2, PB1, PA, NP,
8 M1, M2, NS1, and NS2 proteins in H9N2 viruses isolated from poultry in Egypt. The avian-
9 or mammalian-preference markers are shown.

Viral protein	aa site	Avian preference	Mammalian preference	Egyptian H9N2 (2013-2015)
PB2	44	A	S	A
	64	M	T	M
	81	T	M	T
	199	A	S	A
	318	K	R	K (7), R (3)
	627	E	K	V (8), E (2)
	661	A	T	A
	701	D	N	D
	702	K	R	K
PB1	68	T	I	T
	73	K	R	K (9), E (1)
	76	V	A	V (7), A (3)
	79	R	Q	R (9), Q(1)
	82	L	S	S (8), W (2)
PB1-F2	13	L	P	P
	336	V	I	V
	375	N	S	N
PA	28	P	L	P
	55	D	N	D
	57	R	Q	R
	100	V	A	V (9), A (1)
	133	E	G	E
	225	S	C	S
	241	C	Y	C
	268	L	I	L
	312	K	R	K
	356	K	R	K
	382	E	D	E (8), D (2)
	400	Q/T/S	L	S
	404	A	S	A
	409	S	N	S
	552	T	S	T
	556	Q	R	Q
615	K	L	K (9), R (1)	
NP	31	R	K	R

	33	V	I	V
	34	D	N	G
	61	I	L	I (8),T (2)
	100	R	V	R
	109	I	V	I
	127	E	D	E
	136	L	M	L
	214	R	K	K (8), R (2)
	283	L	P	L
	293	R	K	R
	305	R	K	R
	313	F	Y	F
	357	Q	K	Q
	372	E	D	E
	375	D	G/E	D
	398	K	Q	Q
	422	R	K	R
	442	T	A	T
	455	D	E	D
M1	15	V	I	I
	115	V	I	V
	121	T	A	T
	137	T	A	T
M2	11	T	I	T
	16	E	G/D	G
	20	S	N	S
	28	I	I/V	V (8), I (2)
	57	Y	H	Y
	55	L	F	F
	86	V	A	V
NS1	227	E	K/R	K (8), E (2)

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13 **Table S3:** Hemagglutination inhibition assay titers of monoclonal antibodies against different
 14 Egyptian H9N2 isolates

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Virus	Monoclonal Antibodies			
	Y280	G9	1073	G1
	18G4	(G9-25)	(1073-9)	(G1-26)
A/chicken/Egypt/S4456B/11	800	51200	400	25600
A/chicken/Egypt/D4692A/12	200	204800	800	25600
A/chicken/Egypt/D4905B/12	200	204800	6400	12800
A/chicken/Egypt/S5018C/12	800	25600	800	12800
A/chicken/Egypt/S5018D/12	1600	204800	12800	25600
A/chicken/Egypt/S5440E/12	800	102400	800	12800
A/chicken/Egypt/S5442C/12	400	102400	800	12800
A/chicken/Egypt/S5442E/12	800	102400	3200	25600
A/chicken/Egypt/D5490B/12	400	204800	12800	25600
A/chicken/Egypt/S7022D/13	>100	204800	12800	26500
A/chicken/Egypt/S7025E/13	>100	51200	>100	12800
A/chicken/Egypt/D7099/13	100	204800	800	25600
A/chicken/Egypt/D7100/13	100	204800	400	12800
A/chicken/Egypt/D7108E/13	400	204800	25600	25600
A/chicken/Egypt/S7404/13	400	204800	3200	25600
A/chicken/Egypt/D7436C/13	100	204800	6400	12800
A/chicken/Egypt/S9288A/13	400	25600	>100	6400
A/chicken/Egypt/D9326C/14	800	51200	200	12800
A/chicken/Egypt/S9348B/14	800	51200	400	12800
A/chicken/Egypt/D9377/14	800	51200	200	12800
A/chicken/Egypt/Q9394E/14	800	51200	>100	12800
A/chicken/Egypt/F9516B/14	800	51200	>100	6400
A/chicken/Egypt/D9570B/14	800	25600	800	12800
A/chicken/Egypt/D9817E/14	800	12800	>100	6400
A/quail/Egypt/D9842/14	400	25600	400	200
A/quail/Egypt/D10093/14	400	400	1600	200
A/quail/Egypt/D10105/14	100	100	800	100

A/quail/Egypt/D10106/14	200	200	800	20016
A/chicken/Egypt/F10285D/14	1600	51200	400	12800
A/chicken/Egypt/S10312C/14	800	25600	800	12800
A/pigeon/ Egypt/S10408B/14	800	51200	>100	12800
A/pigeon/ Egypt/S10409A/14	800	51200	>100	12800
A/chicken/Egypt/Q10429E/14	200	12800	400	3200
A/chicken/Egypt/F10534C/15	200	12800	800	3200
A/chicken/Egypt/N10963 /15	400	25600	800	12800
A/chicken/Egypt/N10975/15	200	12800	800	6400
A/chicken/Egypt/F10993B/15	1600	25600	800	12800

Table S4 : Hemagglutination inhibition assay titers of polyclonal antibodies against different Egyptian H9N2 isolates

Antibodies against	A/chicken/Egypt/ D9570B/2014	A/turkey/Israel/1 567/04	A/chicken/Egypt/ D9817E/2014	A/pigeon/ Egypt/S10408B/20 14	A/Hong Kong/1073/99	A/chicken/Egypt/ S4456B/2011	A/quail/leban/27 2/2010	A/Bangladesh/10 994/2011
A/chicken/Egypt/S4456B/11	4096	4096	4096	4096	256	4096	4096	ND
A/chicken/Egypt/D4692A/12	2048	2048	2048	4096	128	4096	1024	256
A/chicken/Egypt/D4905B/12	4096	4096	4096	4096	32	4096	4096	ND
A/chicken/Egypt/S5018D/12	512	512	512	1024	64	1024	256	256
A/chicken/Egypt/S7022D/13	4096	4096	4096	4096	256	4096	1024	ND
A/chicken/Egypt/S7025E/13	4096	4096	4096	4096	64	2048	512	ND
A/chicken/Egypt/D7099/13	4096	4096	4096	4096	256	4096	512	256
A/chicken/Egypt/D7100/13	2048	2048	2048	2048	256	1024	512	256
A/chicken/Egypt/D7108/13	2048	1024	1024	2048	64	1024	256	128
A/chicken/Egypt/S7404/13	1024	1024	1024	1024	128	1024	512	256
A/chicken/Egypt/D7436C/13	1024	1024	1024	2048	128	2048	256	128
A/chicken/Egypt/S9288A/13	1024	1024	1024	1024	32	256	32	2048
A/chicken/Egypt/D9326C/14	1024	1024	2048	2048	64	4096	256	2048
A/chicken/Egypt/S9348B/14	4096	4096	4096	4096	256	4096	4096	4096
A/chicken/Egypt/D9377/14	2048	2048	4096	4096	64	2048	8	2048
A/chicken/Egypt/Q9394E/14	1024	512	1024	1024	32	1024	128	2048
A/chicken/Egypt/F9516B/14	512	512	512	1024	32	256	256	2048
A/chicken/Egypt/D9570B/14	1024	512	2048	1024	32	4096	256	256
A/chicken/Egypt/S9655B/14	4096	4096	4096	4096	256	4096	4096	2048
A/chicken/Egypt/D9817E/14	4069	2048	4096	4096	64	2048	256	1024
A/quail/Egypt/D9842/14	256	512	512	1024	32	1024	256	ND
A/quail/Egypt/D10093/14	32	128	64	128	8	32	64	512
A/quail/Egypt/D10105/14	16	32	16	64	32	8	16	256
A/quail/Egypt/D10106/14	32	128	128	256	32	64	128	256
A/chicken/Egypt/F10285D/14	4096	4096	4096	4069	256	4096	2048	2048
A/chicken/Egypt/S10312C/14	512	512	512	1024	16	1024	256	256

A/pigeon/ Egypt/S10408B/14	2048	512	1024	2048	32	2048	256	2048
A/pigeon/ Egypt/S10409A/14	4096	2048	4096	4096	128	4096	4096	2048
A/chicken/Egypt/Q10429E/14	256	256	512	512	8	512	64	512
A/chicken/Egypt/S10490/15	4096	2048	4096	4096	32	1024	128	ND
A/chicken/Egypt/F10534C/15	128	1024	1024	2048	16	1024	256	2048
A/chicken/Egypt/N10963 /15	4096	2048	4096	4069	128	2048	512	ND
A/chicken/Egypt/N10975/15	2048	1024	1024	2048	128	2048	1024	ND
A/chicken/Egypt/F10993B/15	4096	4096	4096	4096	128	4096	1024	ND

18 **Table S5:** Summarized profile of Egyptian AIV H9N2 isolates used in this study

H9N2 isolate	Governorate	Host	Health status	Sequence		Collection Site	Collection Date
A/chicken/Egypt/S9288A/2013	Sharqia	Chicken	Healthy	HA	NA	Farm	22/12/2013
A/chicken/Egypt/D9326C/2013	Dakahlia	Chicken	Healthy	HA	NA	Farm	28/12/2013
A/chicken/Egypt/S9348B/2014	Sharqia	Chicken	Healthy	HA	NA	House	4/1/2014
A/chicken/Egypt/D9377/2014	Dakahlia	Chicken	Healthy	HA	NA	Market	15/1/2014
A/chicken/Egypt/Q9394E/2014	Qalyubia	Chicken	Dead	HA	NA	Farm	18/1/2014
A/chicken/Egypt/F9516B/2014	Faiyum	Chicken	Sick	Full genome		Farm	15//1/2014
A/chicken/Egypt/D9570B/2014	Dakahlia	Chicken	Healthy	HA	NA	Farm	11/2/2014
A/chicken/Egypt/S9645/2014	Sharqia	Chicken	Healthy	HA	NA	Market	17/2/2014
A/chicken/Egypt/S9652E/2014	Sharqia	Chicken	Healthy	HA	NA	House	17/2/2014
A/chicken/Egypt/S9668D/2014	Sharqia	Chicken	Healthy	HA	NA	Farm	17/2/2014
A/chicken/Egypt/C9742/2014	Cairo	Chicken	Healthy	HA	NA	Market	4/3/2014
A/ duck/Egypt /C9787 /2014	Cairo	Duck	Healthy	Full genome		Market	5/3/2014
A/chicken/Egypt/D9817E/2014	Dakahlia	Chicken	Healthy	HA	NA	Farm	11/3/2014
A/quail/ Egypt /D9842/2014	Dakahlia	Quail	Healthy	Full genome		Market	22/4/2014
A/chicken/Egypt/F9883C/2014	Faiyum	Chicken	Sick	HA	NA	Farm	23/4/2014
A/quail/Egypt/D10093/2014	Dakahlia	Quail	Healthy	Full genome		Market	19/8/2014
A/quail/Egypt/D10105/2014	Dakahlia	Quail	Healthy	Full genome		Market	19/8/2014
A/ quail /Egypt/D10106/2014	Dakahlia	Quail	Healthy	Full genome		Market	19/8/2014
A/chicken/F10285D/2014	Faiyum	Chicken	Dead	HA	NA	Farm	26/10/2014
A/chicken/S10312C/2014	Sharqia	Chicken	Sick	HA	NA	Farm	14/11/2014

A/pigeon/ Egypt/S10408B/2014	Sharqia	Pigeon	Healthy	Full genome		House	9/12/2014
A/pigeon/Egypt/S10409A/2014	Sharqia	Pigeon	Healthy	Full genome		House	9/12/2014
A/chicken/Egypt/Q10429E/2014	Qalyubia	Chicken	Dead	Full genome		Farm	13/12/2014
A/chicken/Egypt/S10489C/2015	Sharqia	Chicken	Healthy	HA	NA	Farm	5/1/2015
A/chicken/Egypt/S10490/2015	Sharqia	Chicken	Healthy	HA	NA	Market	5/1/2015
A/chicken/Egypt/F10528A/2015	Faiyum	Chicken	Sick	HA	NA	Farm	19/1/2015
A/chicken/Egypt/F10533A/2015	Faiyum	Chicken	Sick	HA	NA	Farm	19/1/2015
A/chicken/Egypt/F10533D/2015	Faiyum	Chicken	Sick	HA	NA	Farm	19/1/2015
A/chicken/Egypt/F10534C/2015	Faiyum	Chicken	Sick	HA	NA	Farm	19/1/2015
A/chicken/Egypt/D10549D/2015	Dakahlia	Chicken	Sick	HA	NA	Farm	27/1/2015
A/chicken/Egypt/D10560/2015	Dakahlia	Chicken	Healthy	HA	NA	Market	27/1/2015
A/chicken/Egypt/D10561/2015	Dakahlia	Chicken	Healthy	HA	NA	Market	27/1/2015
A/chicken/Egypt/D10562/2015	Dakahlia	Chicken	Healthy	HA	NA	Market	27/1/2015
A/chicken/Egypt/D10564/2015	Dakahlia	Chicken	Healthy	HA	NA	Market	27/1/2015
A/chicken/Egypt/D10566B/2015	Dakahlia	Chicken	Sick	HA	NA	Farm	27/1/2015
A/chicken/Egypt/A10588/2015	Asyut	Chicken	Healthy	HA	NA	Market	9/2/2015
A/chicken/Egypt/A10593D/2015	Asyut	Chicken	Dead	HA	NA	House	9/2/2015
A/chicken/Egypt/S10597A/2015	Sharqia	Chicken	Sick	HA	NA	Farm	15/2/2015
A/chicken/Egypt/S10598E/2015	Sharqia	Chicken	Sick	HA	NA	Farm	15/2/2015
A/chicken/Egypt/Q10649C/2015	Qalyubia	Chicken	Dead	HA	NA	Farm	18/2/2015
A/chicken/Egypt/Q10660B/2015	Qalyubia	Chicken	Dead	HA	NA	Farm	21/2/2015
A/chicken/Egypt/D10685D/2015	Dakahlia	Chicken	Healthy	HA	NA	Farm	26/2/2015

A/chicken/Egypt/D10700/2015	Dakahlia	Chicken	Healthy	HA	NA	Market	26/2/2015
A/chicken/Egypt/D10704/2015	Dakahlia	Chicken	Healthy	HA	NA	Market	26/2/2015
A/chicken/Egypt/D10705/2015	Dakahlia	Chicken	Healthy	HA	NA	Market	26/2/2015
A/chicken/Egypt/D10706E/2015	Dakahlia	Chicken	Sick	HA	NA	Farm	26/2/2015
A/chicken/Egypt/D10707B/2015	Dakahlia	Chicken	Sick	HA	NA	Farm	26/2/2015
A/chicken/Egypt/D10788/2015	Dakahlia	Chicken	Healthy	HA	NA	Market	21/3/2015
A/chicken/Egypt/D10789/2015	Dakahlia	Chicken	Healthy	HA	NA	Market	21/3/2015
A/chicken/Egypt/D10798D/2015	Dakahlia	Chicken	Healthy	HA	NA	Farm	21/3/2015
A/chicken/Egypt/D10799A/2015	Dakahlia	Chicken	Healthy	HA	NA	Farm	21/3/2015
A/chicken/Egypt/D10802E/2015	Dakahlia	Chicken	Healthy	HA	NA	Farm	28/3/2015
A/chicken/Egypt/S10916B/2015	Sharqia	Chicken	Healthy	HA	NA	Farm	6/4/2015
A/chicken/Egypt/D10939E/2015	Dakahlia	Chicken	Healthy	HA	NA	Farm	20/4/2015
A/chicken/Egypt/D10945E/2015	Dakahlia	Chicken	Healthy	HA	NA	Farm	20/4/2015
A/chicken/Egypt/N10957A/2015	Minya	Chicken	Healthy	HA	NA	Farm	24/4/2015
A/chicken/Egypt/N10963 /2015	Minya	Chicken	Healthy	HA	NA	Abattoir	24/4/2015
A/chicken/Egypt/N10975/2015	Minya	Chicken	Healthy	HA	NA	Market	24/4/2015
A/chicken/Egypt/F10993B/2015*	Faiyum	Chicken	Dead	Full genome		Farm	28/4/2015

19 *This isolate was obtained from vaccinated poultry by H9N2 vaccine (Ceva).

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23 **Table S6:** Summarized Egyptian AIV H9N2 genes released in this study with their accession
 24 numbers in GenBank

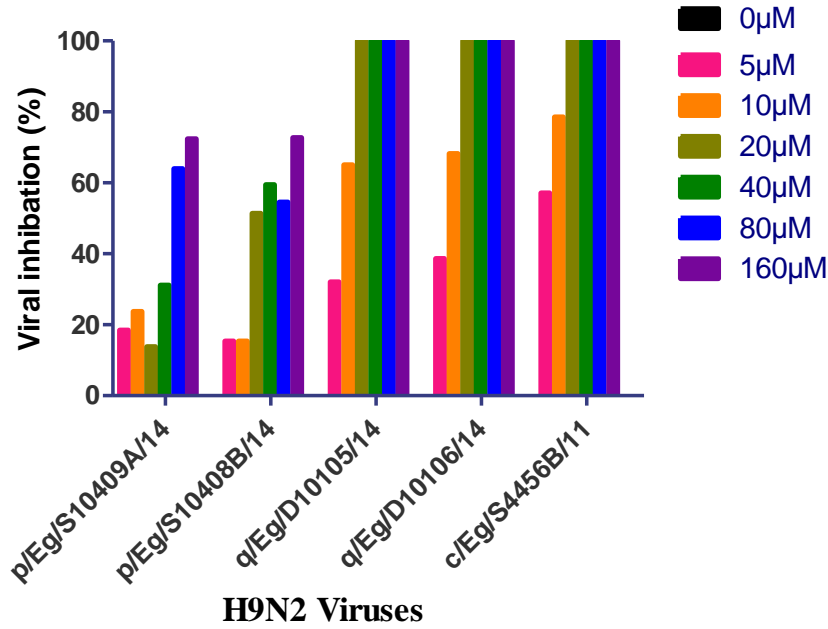
25

Isolate	PB2	PB1	PA	HA	NP	NA	M	NS
A/chicken/Egypt/S9288A/2013				KX000736		KX000773		
A/chicken/Egypt/D9326C/2013				KX000797		KX000868		
A/chicken/Egypt/S9348B/2014				KX000748		KX000803		
A/chicken/Egypt/D9377/2014				KX000845		KX000851		
A/chicken/Egypt/Q9394E/2014				KX000735		KX000799		
A/chicken/Egypt/F9516B/2014	KX000870	KX000762	KX000776	KX000718	KX000765	KX000831	KX000835	KX000709
A/chicken/Egypt/D9570B/2014				KX000817		KX000786		
A/chicken/Egypt/S9645/2014				KX000758		KX000746		
A/chicken/Egypt/S9652E/2014				KX000823		KX000768		
A/chicken/Egypt/S9668D/2014				KX000754		KX000829		
A/chicken/Egypt/C9742/2014				KX000864		KX000795		
A/ duck/Egypt /C9787 /2014	KX000804	KX000841	KX000755	KX000751	KX000767	KX000738	KX000728	KX000747
A/chicken/Egypt/D9817E/2014				KX000871		KX000840		
A/quail/ Egypt /D9842/2014	KX000720	KX000869	KX000740	KX000857	KX000733	KX000858	KX000771	KX000725
A/chicken/Egypt/F9883C/2014				KX000860		KX000879		
A/quail/Egypt/D10093/2014	KX000731	KX000772	KX000730	KX000790	KX000818	KX000872	KX000833	KX000724
A/quail/Egypt/D10105/2014	KX000784	KX000711	KX000800	KX000710	KX000809	KX000852	KX000819	KX000780
A/ quail /Egypt/D10106/2014	KX000827	KX000826	KX000737	KX000865	KX000810	KX000722	KX000834	KX000705
A/chicken/F10285D/2014				KX000713		KX000850		
A/chicken/S10312C/2014				KX000760		KX000832		
A/pigeon/ Egypt/S10408B/2014	KX000876	KX000837	KX000855	KX000789	KX000866	KX000811	KX000779	KX000859
A/pigeon/Egypt/S10409A/2014	KX000764	KX000729	KX000723	KX000715	KX000806	KX000778	KX000828	KX000785
A/chicken/Egypt/Q10429E/2014	KX000752	KX000877	KX000874	KX000830	KX000744	KX000856	KX000714	KX000849
A/chicken/Egypt/S10489C/2015				KX000794		KX000757		
A/chicken/Egypt/S10490/2015				KT216664		KX000842		
A/chicken/Egypt/F10528A/2015				KX000801		KX000753		
A/chicken/Egypt/F10533A/2015				KX000712		KX000808		
A/chicken/Egypt/F10533D/2015				KX000745		KX000848		
A/chicken/Egypt/F10534C/2015				KX000763		KX000721		
A/chicken/Egypt/D10549D/2015				KX000863		KX000774		
A/chicken/Egypt/D10560/2015				KX000741		KX000854		
A/chicken/Egypt/D10561/2015				KX000844		KX000875		
A/chicken/Egypt/D10562/2015				KX000770		KX000796		
A/chicken/Egypt/D10564/2015				KX000822		KX000873		

A/chicken/Egypt/D10566B/2015				KX000878		KX000816		
A/chicken/Egypt/A10588/2015				KX000814		KX000759		
A/chicken/Egypt/A10593D/2015				KX000846		KX000717		
A/chicken/Egypt/S10597A/2015				KX000708		KX000727		
A/chicken/Egypt/S10598E/2015				KX000792		KX000812		
A/chicken/Egypt/Q10649C/2015				KX000732		KX000787		
A/chicken/Egypt/Q10660B/2015				KX000775		KX000761		
A/chicken/Egypt/D10685D/2015				KX000766		KX000726		
A/chicken/Egypt/D10700/2015				KX000756		KX000734		
A/chicken/Egypt/D10704/2015				KX000821		KX000798		
A/chicken/Egypt/D10705/2015				KX000861		KX000742		
A/chicken/Egypt/D10706E/2015				KX000815		KX000820		
A/chicken/Egypt/D10707B/2015				KX000836		KX000862		
A/chicken/Egypt/D10788/2015				KX000805		KX000813		
A/chicken/Egypt/D10789/2015				KX000739		KX000824		
A/chicken/Egypt/D10798D/2015				KX000716		KX000782		
A/chicken/Egypt/D10799A/2015				KX000777		KX000719		
A/chicken/Egypt/D10802E/2015				KT216663		KT216662		
A/chicken/Egypt/S10916B/2015				KX000853		KX000807		
A/chicken/Egypt/D10939E/2015				KX000750		KX000839		
A/chicken/Egypt/D10945E/2015				KX000793		KX000707		
A/chicken/Egypt/N10957A/2015				KX000781		KX000788		
A/chicken/Egypt/N10963 /2015				KX000838		KX000802		
A/chicken/Egypt/N10975/2015				KX000743		KX000749		
A/chicken/Egypt/F10993B/2015	KX000706	KX000769	KX000825	KX000791	KX000867	KX000843	KX000847	KX000783

27 **Figure S1.** Antiviral sensitivity assays for Egyptian H9N2 viruses to amantadine with IC₅₀ (a) and
 28 oseltamivir (b) were determined in MDCK cells by plaque reduction assay
 29

30 **Fig. S1a**



H9N2 Virus	IC ₅₀ (μm of amantadine)
A/pigeon/Egypt/S10409A/2014	110
A/pigeon/ Egypt/S10408B/2014	70
A/quail/Egypt/D10105/2014	8.2
A/quail/Egypt/D10106/2014	7
A/chicken/Egypt/S4456B/2011	1.4

49 **Fig. S1b**

