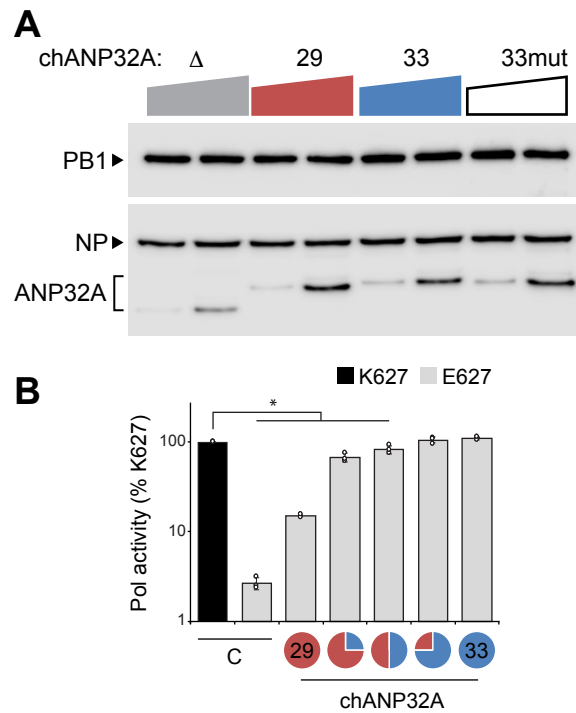


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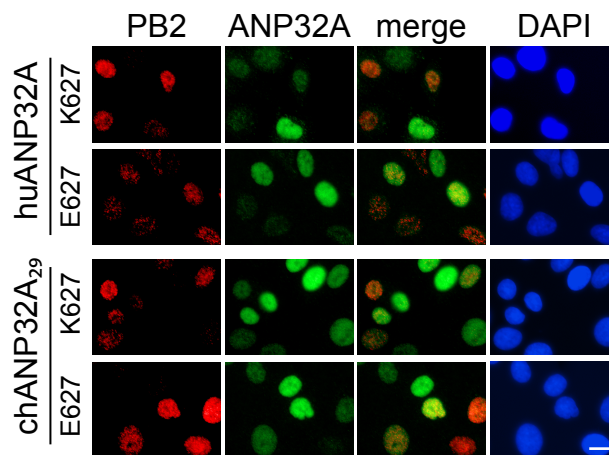
**Supplemental Information**

**Differential Splicing of ANP32A in Birds  
Alters Its Ability to Stimulate RNA Synthesis  
by Restricted Influenza Polymerase**

**Steven F. Baker, Mitchell P. Ledwith, and Andrew Mehle**



**Figure S1. Comparison of ANP32A isoform expression levels and compatibility. Related to Figure 2.** (A) ANP32A variants are expressed at similar levels. Samples from Fig 2D containing the two highest amounts of each ANP32A variant were re-run on the same gel to enable comparison. Proteins were detected by western blot. (B) PB2 E627 polymerase activity is enhanced when the predominant avian ANP32A isoforms are expressed alone or in combination. Total amounts of chANP32A added to the PB2 E627 polymerase activity assays remained constant, while the proportions of chANP32A<sub>29</sub> and chANP32A<sub>33</sub> were varied as represented in the pie charts. Data are shown as means (columns) of  $n = 3$  technical replicates (dots)  $\pm$  SD. Representative results of at least 3 independent biological replicates. C = empty vector control. \* $p < 0.05$ , (Student's T-test) between PB2 K627 and E627 at each condition.



**Figure S2. PB2 and ANP32A localize to the nucleus. Related to Figure 2.** A549 cells were transduced with the indicated ANP32A and infected with PB2 K627 or PB2 E627 WSN (MOI, 1) for 5 h. PB2 (FLAG) and ANP43A (V5) were detected by Immunofluorescence. Nuclei were identified by DAPI staining. Scale bar, 10  $\mu$ m.

Table S1. Data underlying graphics presented in Fig 1B-D with references to original source sequencing files. Related to Figure 1.

NCBI Reference Sequence	Region	RNA-Seq SRA		Phylogenetic tree branch	Genus species	Common name	notes	+VLSL			-VLSL			ex 5 skipping ex 5-6			
		GeneID no.	ID no.					reads	%	reads	%	reads	reads	%			
NW_009270572.1	237801-247220	104139231	SRR504696	Ostrich	Struthio camelus australis	South African ostrich	<i>no insert/repeat</i>										<b>100</b>
NW_014000784.1	62380-87756	106492272	ERR522068	Tinamous & allies	Apteryx australis mantelli	North Island brown kiwi	<i>no insert/repeat</i>										<b>100</b>
NW_013185803.1	860304-876465	106043638	SRR1796005	Waterfowl	Anser cygnoides domesticus	Swan goose		226	<b>26</b>	12	<b>1</b>	525	201	<b>72</b>			
NW_004677346.1	204119-216117	101805403	SRR1796027	Waterfowl	Anas platyrhynchos	Mallard		374	<b>88</b>	45	<b>11</b>	9	666	<b>1</b>			
NC_034417.1	18981267-19002520	110403929	SRR1795827	Landfowl	Numida meleagris	Helmeted guineafowl		262	<b>85</b>	47	<b>15</b>						
NW_015438805.1	1706835-1724257	107318936	SRR5912687	Landfowl	Coturnix japonica	Japanese quail		28	<b>57</b>	12	<b>24</b>	5	22	<b>19</b>			
NT_455925.1	1152927-1173155	415562	SRR5823193	Landfowl	Gallus gallus	Red junglefowl		171	<b>77</b>	51	<b>23</b>						
NC_015022.2	18498947-18516371	100547535	SRR1796077	Landfowl	Meleagris gallopavo	Wild turkey		299	<b>74</b>	105	<b>26</b>						
NW_004973680.1	4318444-4340310	102093694	SRR5878854	Doves	Columba livia	Rock pigeon		3922	<b>95</b>	68	<b>2</b>	102	2854	<b>3</b>			
NW_015090969.1	1121007-1145953	106894196	ERR1018134	Shorebirds	Calidris pugnax	Ruff		277	<b>77</b>	12	<b>3</b>	67	279	<b>19</b>			
			SRR3218026	Shorebirds	Leucophaeus atricilla	Laughing gull		23	<b>100</b>	0	<b>0</b>						
NW_008795371.1	4618727-4683148	103899909	SRR1693186	Penguins	Aptenodytes forsteri	Emperor penguin		43	<b>55</b>	0	<b>0</b>	30	37	<b>45</b>			
NW_011950872.1	22765800-22788757	105412387	SRR1817947	Eagles, Hawks	Aquila chrysaetos canadensis	American golden eagle		268	<b>100</b>	0	<b>0</b>						
NW_005087564.1	19249592-19262951	102112109	SRR768235	Passerines	Pseudopodoces humilis	Ground tit		23	<b>56</b>	18	<b>44</b>						
NC_031779.1	19145635-19150648	107209139	SRR3955331	Passerines	Parus major	Great tit		25	<b>32</b>	54	<b>68</b>						
NW_014650706.1	1048593-1060262	106860135	SRR3990507	Passerines	Sturnus vulgaris	Common starling	ALSL	7	<b>7</b>	94	<b>93</b>						
NW_007931134.1	16689454-16696031	103816257	SRR2915372	Passerines	Serinus canaria	Common canary	ALSL	835	<b>68</b>	392	<b>32</b>						
NW_017219526.1	1317935-1327225	103757016	SRR3476292	Passerines	Manacus vitellinus	Golden-collared manakin		2416	<b>79</b>	312	<b>10</b>	397	3105	<b>11</b>			
NW_016690356.1	99142-119184	108504826	SRR3493972	Passerines	Lepidothrix coronata	Blue-crowned manakin		1745	<b>76</b>	237	<b>10</b>	391	2400	<b>14</b>			
NW_018113967.1	5243961-5249928	104684065	SRR1947400	Passerines	Corvus cornix cornix	Hooded crow		125	<b>57</b>	96	<b>43</b>						
NW_018657095.1	109336-132945	110477559	SRR5223631	Passerines	Lonchura striata domestica	Bengalese finch		1325	<b>34</b>	1888	<b>48</b>	620	2767	<b>18</b>			
NW_002197120.1	1-14870	100190140	SRR2545949	Passerines	Taeniopygia guttata	Zebra finch		56	<b>88</b>	8	<b>13</b>						

NCBI Reference Sequence	Region	RNA-Seq SRA		Higher taxon name	RNA source	Treatment	notes	+VLSL			-VLSL			ex 5 skipping ex 5-6		
		GeneID no.	ID no.					reads	%	reads	%	reads	reads	%		
NT_455925.1	1152927-1173155	415562	SRR2072645	Galliformes	DF1 chicken fibroblast cells	mock, empty DNA		304	<b>83</b>	63	<b>17</b>					
NT_455925.1	1152927-1173155	415562	SRR2072646	Galliformes	DF1 chicken fibroblast cells	pl:C, empty DNA		617	<b>82</b>	136	<b>18</b>					
NT_455925.1	1152927-1173155	415562	SRR2072647	Galliformes	DF1 chicken fibroblast cells	mock, IRF7 DNA		637	<b>78</b>	175	<b>22</b>					
NT_455925.1	1152927-1173155	415562	SRR2072648	Galliformes	DF1 chicken fibroblast cells	pl:C IRF7 DNA		379	<b>83</b>	80	<b>17</b>					
NT_455925.1	1152927-1173155	415562	SRR2072649	Galliformes	DF1 chicken fibroblast cells	mock, sh-control		509	<b>76</b>	163	<b>24</b>					
NT_455925.1	1152927-1173155	415562	SRR2072650	Galliformes	DF1 chicken fibroblast cells	pl:C, sh-control		216	<b>74</b>	74	<b>26</b>					
NT_455925.1	1152927-1173155	415562	SRR2072651	Galliformes	DF1 chicken fibroblast cells	mock, shIRF7		492	<b>75</b>	165	<b>25</b>					
NT_455925.1	1152927-1173155	415562	SRR2072652	Galliformes	DF1 chicken fibroblast cells	pl:C, shIRF7		249	<b>77</b>	76	<b>23</b>					
NT_455925.1	1152927-1173155	415562	SRR1284638	Galliformes	Leghorn chicken (susceptible)	mock		216	<b>81</b>	52	<b>19</b>					
NT_455925.1	1152927-1173155	415562	SRR1284637	Galliformes	Leghorn chicken (susceptible)	infected, influenza H5N3		233	<b>75</b>	79	<b>25</b>					
NT_455925.1	1152927-1173155	415562	SRR1284640	Galliformes	Fayoumi chicken (resistant)	mock		144	<b>80</b>	35	<b>20</b>					
NT_455925.1	1152927-1173155	415562	SRR1284639	Galliformes	Fayoumi chicken (resistant)	infected, influenza H5N3		130	<b>72</b>	51	<b>28</b>					
NC_000015.10	68778535-68820922	8125	SRR5458553	Mammalia	A549 human lung epithelial cells	mock	<i>no insert/repeat</i>									<b>100</b>
NC_000015.10	68778535-68820922	8125	SRR5458559	Mammalia	A549 human lung epithelial cells	infected, influenza H7N9	<i>no insert/repeat</i>									<b>100</b>

required modification