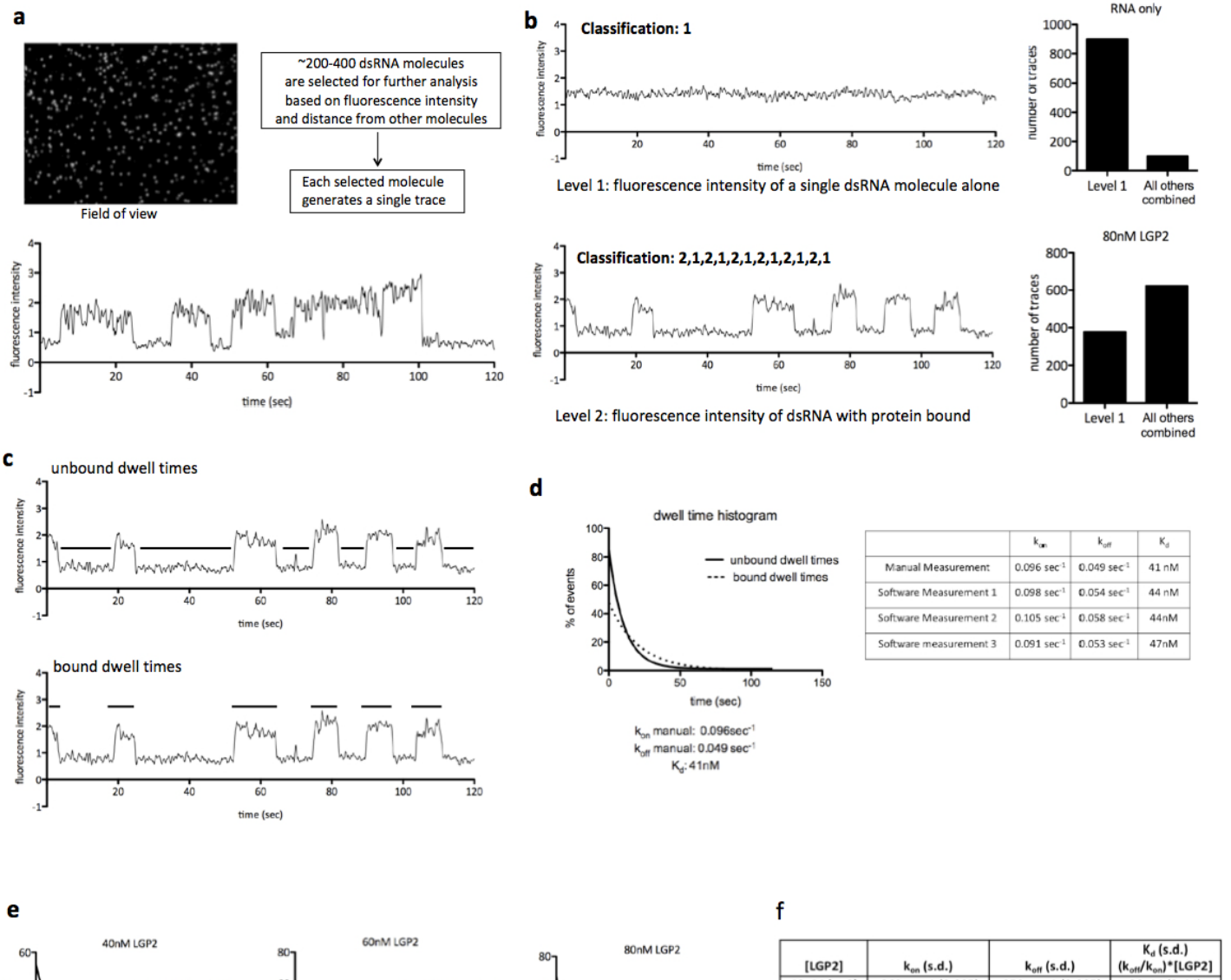
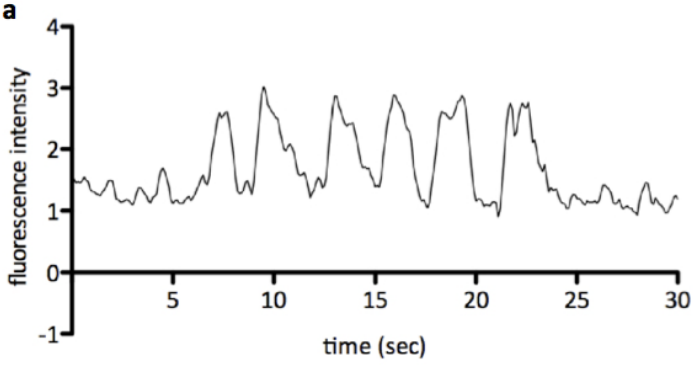


	k_{on}	k_{off}	K_d
Manual Measurement	0.096 sec^{-1}	0.049 sec^{-1}	41 nM
Software Measurement 1	0.098 sec^{-1}	0.054 sec^{-1}	44 nM
Software Measurement 2	0.105 sec^{-1}	0.058 sec^{-1}	44nM
Software measurement 3	0.091 sec^{-1}	0.053 sec^{-1}	47nM

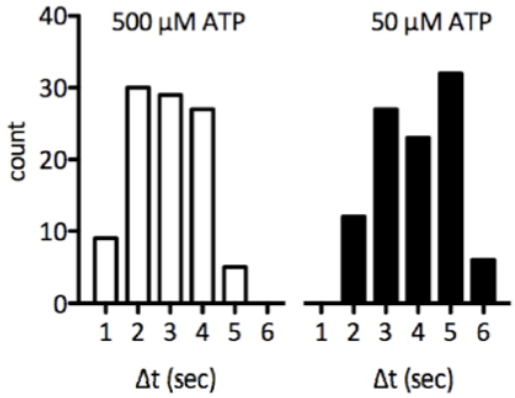
f

[LGP2]	k_{on} (s.d.)	k_{off} (s.d.)	K_d (s.d.) (k_{off}/k_{on})*[LGP2]
40nM (n=3)	.069 sec-1 (0.005)	.050 sec-1 (0.005)	29nM (2nM)
60nM (n=3)	.082 sec-1 (0.005)	.053 sec-1 (0.004)	39nM (3nM)
80nM (n=6)	.094 sec-1 (0.015)	.059 sec-1 (0.007)	51nM (7nM)

a

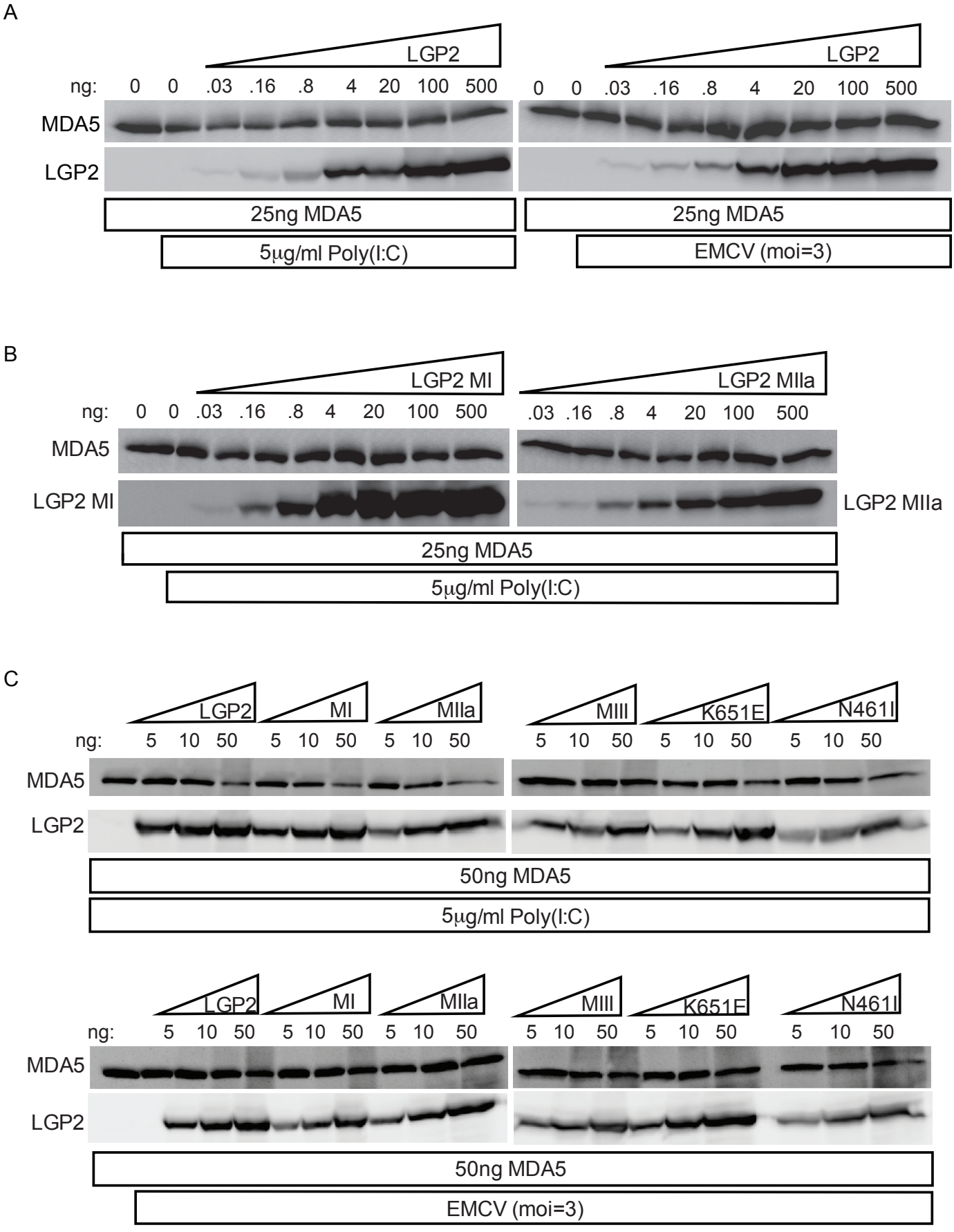


b



c

	Bruns, et al. 2012	Myong, et al. 2009
K_d	98nM ± 13nM	76.3nM ± 6.9nM
Δt at 50/60 µM ATP	4.0 sec	3.7 sec
Δt at 100 µM ATP	n.d.	2.8 sec
Δt at 500 µM ATP	2.9 sec	n.d.


A
LGP2
LGP2
ng: 0 0 .03 .16 .8 4 20 100 500
0 0 .03 .16 .8 4 20 100 500
MDA5
LGP2
25ng MDA5
25ng MDA5
5μg/ml Poly(I:C)
EMCV (moi=3)
B
LGP2 MI
LGP2 MIIa
ng: 0 0 .03 .16 .8 4 20 100 500
.03 .16 .8 4 20 100 500
MDA5
LGP2 MI
LGP2 MIIa
25ng MDA5
5μg/ml Poly(I:C)
C
LGP2
MI
MIIa
MIII
K651E
N461I
ng: 5 10 50 5 10 50 5 10 50 5 10 50 5 10 50 5 10 50
MDA5
LGP2
50ng MDA5
5μg/ml Poly(I:C)
LGP2
MI
MIIa
MIII
K651E
N461I
ng: 5 10 50 5 10 50 5 10 50 5 10 50 5 10 50 5 10 50
MDA5
LGP2
50ng MDA5
EMCV (moi=3)

Supplemental Figure Legends

Figure S1. Data analysis. (A) Custom written software selects molecules for analysis based on their individual fluorescence intensity and their proximity to other molecules. Each selected molecule generates a single trace of fluorescence intensity over time, with one frame captured every 100 milliseconds. Approximately 200-400 RNA molecules are analyzed per field of view and the traces from 6-10 fields of view are compiled for each experimental condition. (B) The software then classifies the fluorescence intensities as level 1 corresponding to RNA alone, level 2 is RNA with protein bound, and level 0 represents a bleached fluorophore. In ~1000 traces captured of RNA alone, approximately 900 traces have fluorescence intensity corresponding to level 1 only (upper graph). When 80nM LGP2 protein is added, the number of traces with level 2 fluorescence intensity is greatly increased (lower graph). (C) A second custom-written program then generates histograms based on the dwell times in the unbound or bound state, before a binding or unbinding event occurs, respectively. These dwell time histograms are fit to an exponential decay form $y=y_0\ e^{-kx}$, where k determines the on or off rate. (D) Data presented are one manual measurement of the k_{on}, k_{off}, and K_d values at 80nM LGP2 compared to three independent experiments of 80nM LGP2 measured by the dwell time analysis software. Histograms of dwell times measured manually verify the accuracy of the software in determining bound and unbound lifetimes, resulting in similar k_{on}, k_{off}, and K_d values. (E) On and off rate histograms at varying concentrations of LGP2 protein generated by dwell time analysis software.

Values shown are taken from one representative experiment. (F) Average k_{on}, k_{off}, and K_d values from at least three independent experiments (n). Experiment demonstrates that k_{on} increases with protein concentration, while k_{off} is relatively constant. Standard deviation is shown in parentheses.

Figure S2. Translocation of RIG-I-C. (A) Representative trace depicting RIG-I-C translocation at 500 μM ATP. (B) The time between peak fluorescence intensities, or the period of translocation labeled as Δt. The distribution of periods of translocation at 50 and 500 μM ATP demonstrates the rate of translocation is dependent on the concentration of ATP. (C) Comparison of RIG-I-C K_d and periods of translocation between Myong et al. 2009, and Bruns et al. 2012. n.d.=not determined.

Figure S3. MDA5 and LGP2 expression levels from luciferase assays. Protein extracts from luciferase assays were separated by SDS-PAGE, transferred to nitrocellulose, and probed with Flag-HRP antibody to verify LGP2 expression levels, and probed with a mouse monoclonal MDA5 antibody (3B11). Panels A, B, and C correspond to luciferase assays done in panels A, B, and C of figure 4. MDA5 levels are constant across a single experiment, while LGP2 levels increase with increasing transfected plasmid.