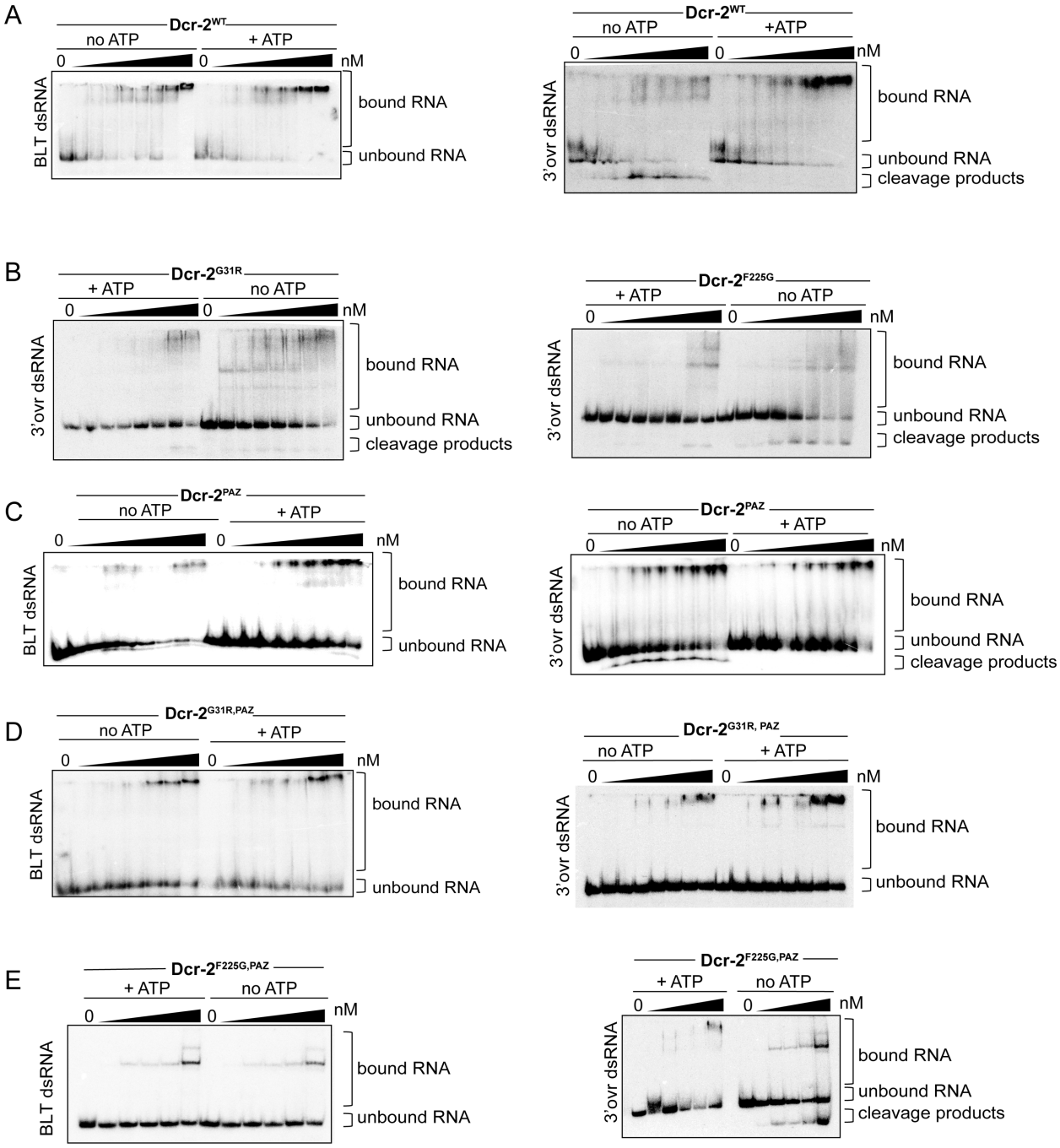
A
Dcr-2WT
no ATP
+ ATP
0
0
nM
BLT dsRNA
bound RNA
unbound RNA
Dcr-2WT
no ATP
+ATP
0
0
nM
3'ovr dsRNA
bound RNA
unbound RNA
cleavage products
B
Dcr-2G31R
+ ATP
no ATP
0
0
nM
3'ovr dsRNA
bound RNA
unbound RNA
cleavage products
Dcr-2F225G
+ ATP
no ATP
0
0
nM
3'ovr dsRNA
bound RNA
unbound RNA
cleavage products
C
Dcr-2PAZ
no ATP
+ ATP
0
0
nM
BLT dsRNA
bound RNA
unbound RNA
Dcr-2PAZ
no ATP
+ ATP
0
0
nM
3'ovr dsRNA
bound RNA
unbound RNA
cleavage products
D
Dcr-2G31R,PAZ
no ATP
+ ATP
0
0
nM
BLT dsRNA
bound RNA
unbound RNA
Dcr-2G31R, PAZ
no ATP
+ ATP
0
0
nM
3'ovr dsRNA
bound RNA
unbound RNA
E
Dcr-2F225G,PAZ
+ ATP
no ATP
0
0
nM
BLT dsRNA
bound RNA
unbound RNA
Dcr-2F225G,PAZ
+ ATP
no ATP
0
0
nM
3'ovr dsRNA
bound RNA
unbound RNA
cleavage products

S5: Primary EMSA data for Dcr-2 wildtype and variants**.** PhosphorImages show representative EMSAs for Dcr-2 enzymes as indicated, using 20pM ^{32}P-labeled 52- BLT dsRNA or 3'ovr dsRNA as indicated. Dcr-2 concentrations used, from left to right, were A) 0, 10, 25, 50, 75, 100, 150, 200, 400nM Dcr-2^{WT} for both left and right gel. B) 0, 10, 25, 50, 75, 100, 150, 400nM Dcr-2^{G31R} (left); 0, 25, 50, 70, 100, 200, 400, 800nM Dcr-2^{F225G} (right). C) 0, 10, 25, 50, 60, 75, 100, 200, 400nM Dcr-2^{PAZ} (left); 0, 25, 50, 75, 100, 175, 200, 275, 400nM Dcr-2^{PAZ} (right). D) 0, 25, 50, 75, 100, 200, 400, 530nM Dcr-2^{G31R,PAZ} (left); 0, 10, 25, 50, 60, 75, 200, 400nM Dcr-2^{G31R,PAZ} (right). E) 0, 50, 75, 100, 260, 420nM Dcr-2^{F225G,PAZ} (left); 0, 25, 75, 100, 260, 420nM Dcr-2^{F225G,PAZ} (right). Dcr-2^{F225G,PAZ} was only purified through Strep-Tactin affinity chromatography. All Dicer proteins contained a wildtype RNase III domain, and some assays showed cleavage products (labeled), which were included in bound RNA. Control experiments using proteins with mutations in the RNase III domains showed only insignificant differences in calculated Kd values.