

Supplementary files

RNA Sequencing revealed differentially expressed genes functionally associated with immunity and tumor suppression during latent phase infection of a vv+MDV in chickens

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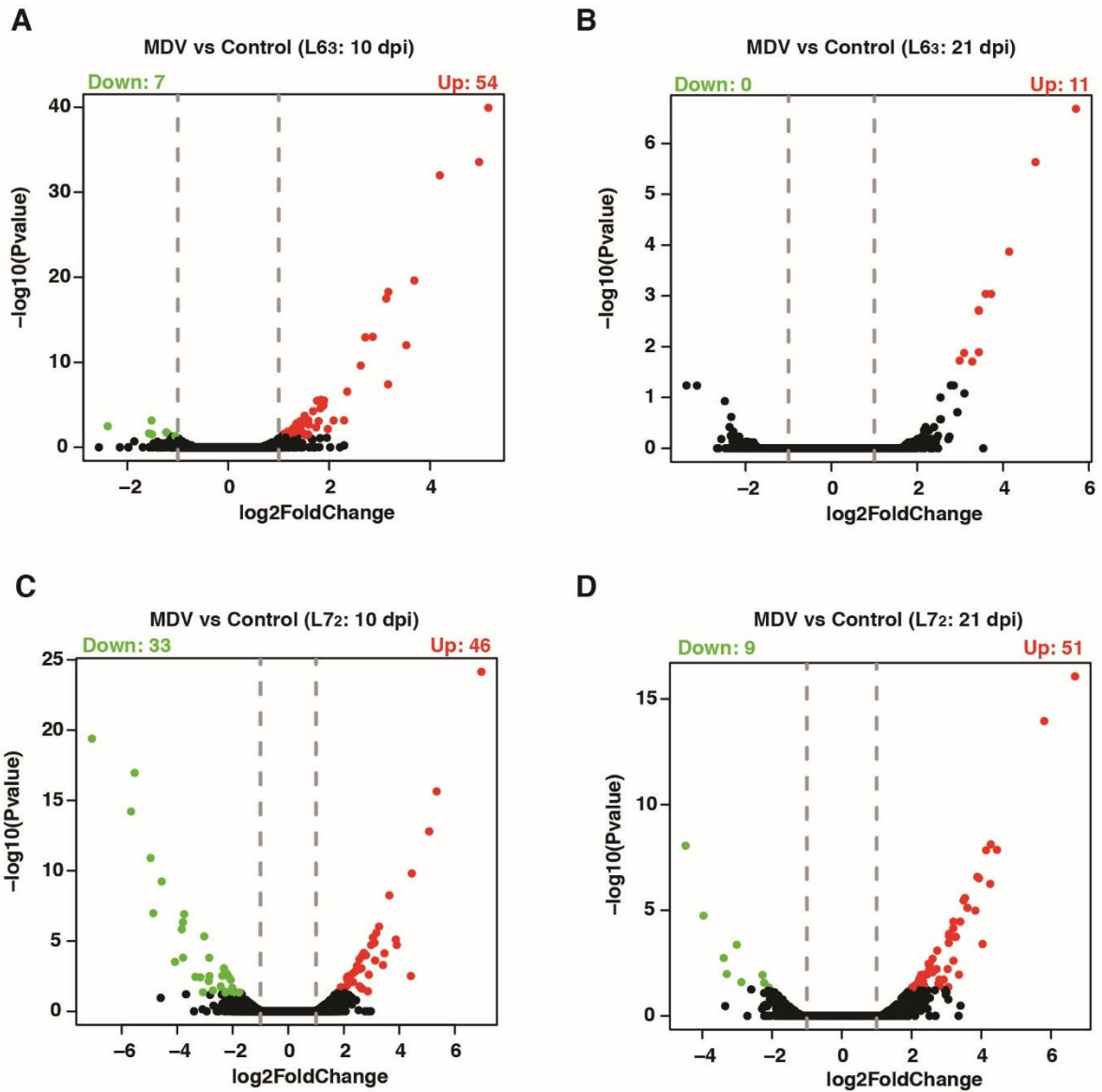


Figure S1. Differentially expressed genes in response to MDV challenge identified in line 6₃ and line 7₂ at 10 and 21 dpi. Volcano plots showing the differentially expressed genes in response to MDV challenge in line 6₃ at 10 dpi (A) and 21 dpi (B); in line 7₂ at 10 dpi (C) and 21 dpi (D).

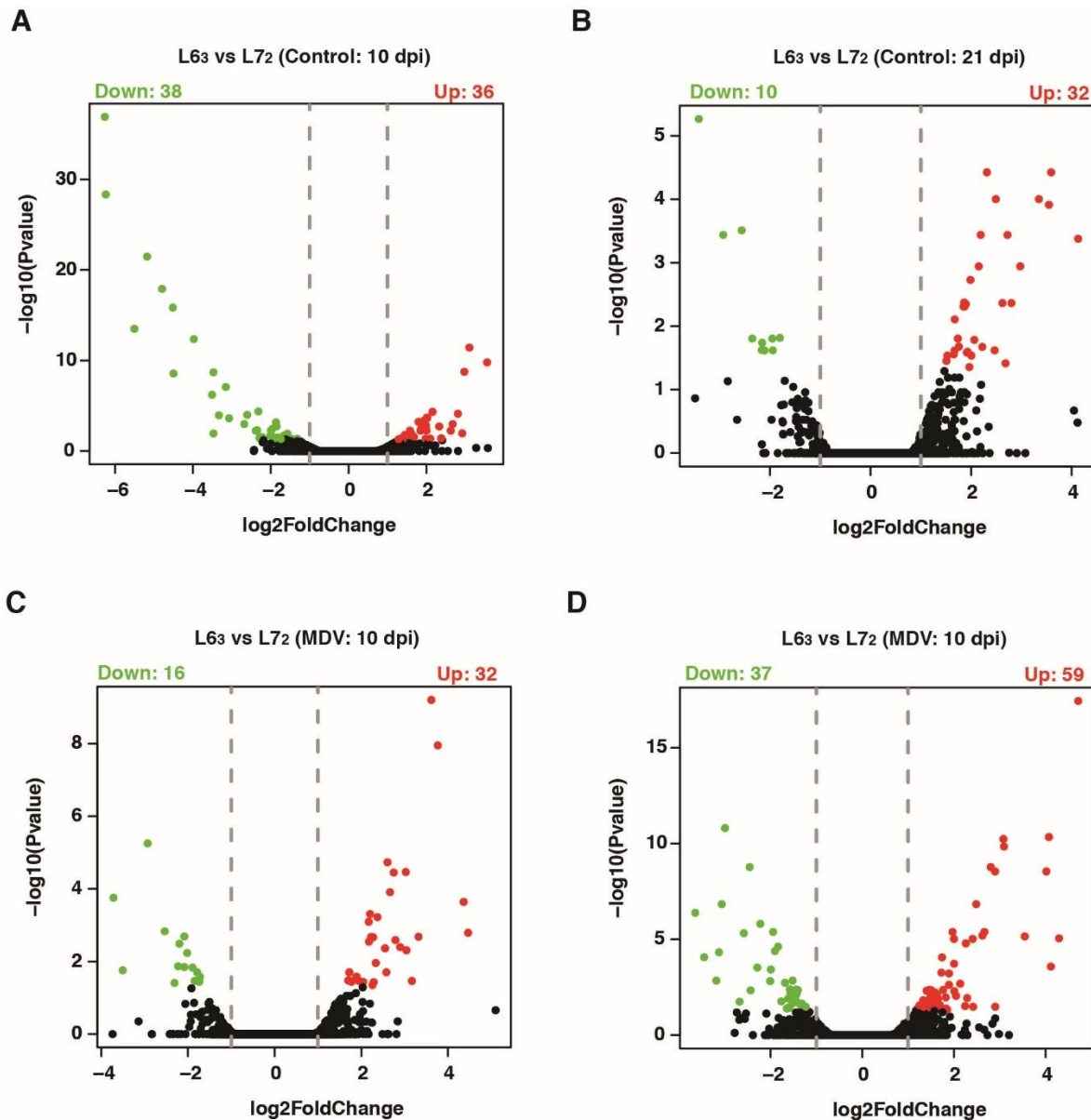


Figure S2. Differentially expressed genes between non-challenged and challenged line 63 and line 72 at 10 and 21 dpi. Volcano plots showing the differentially expressed genes between non-challenged line 63 and line 72 birds at 10 dpi (A) and 21 dpi (B); and between challenged line 63 and line 72 at 10 dpi (C) and 21 dpi (D).

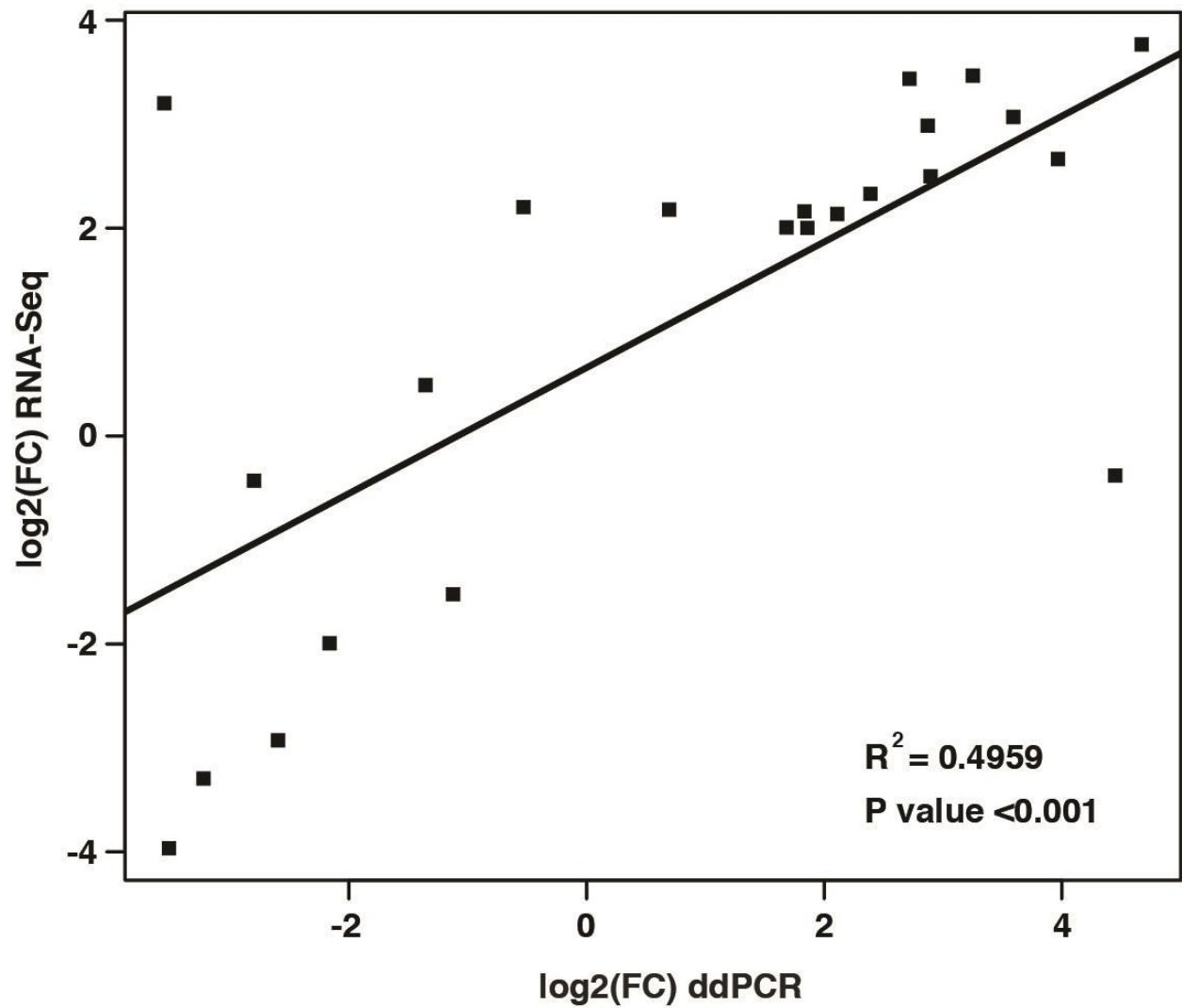
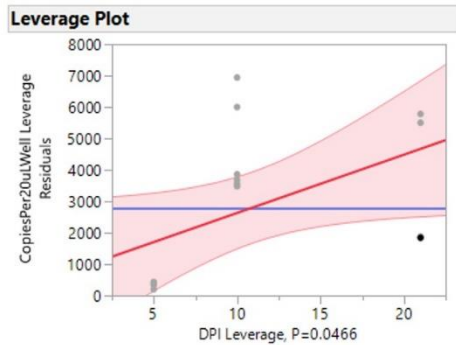


Figure S3. A plot showing correlation between the expression levels of a selected subgroup of genes identified by RNA-Seq and re-tested with ddPCR. The overall correlation coefficient for the selected subgroup of genes in test was $r = 0.7$, which validated the expression data acquired by RNA-Seq with a reasonable confidence.

Line 6₃ total RNA samples



Line 7₂ total RNA samples

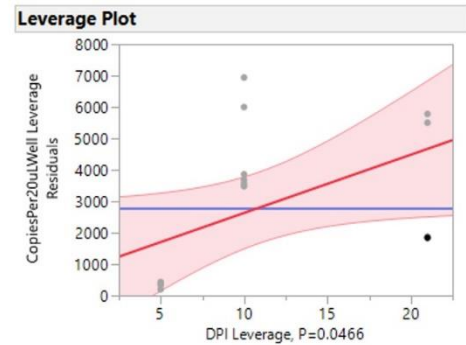
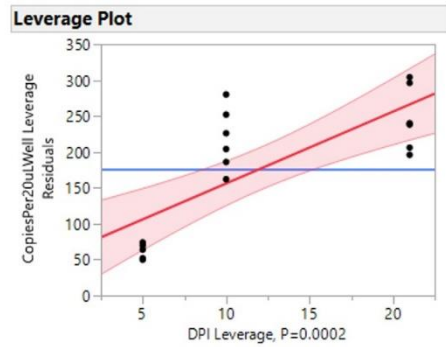


Figure S4. MDV miR-M3-3p microRNA expression leverage plots based on ddPCR absolute quantifications of the microRNA copies per 20- μ l reaction at 5, 10 and 21 days post MDV challenge for each individual sample with technical replicates (the 5 dpi total RNA samples were borrowed from a sister project, which was conducted simultaneously under the exact same conditions using the same batch of line 6₃ and 7₂ chicks). It is clearly shown that the miR-M3-3p microRNA expressions of all birds at 10 and 21 dpi differed from the expressions at 5 dpi in both line 6₃ and line 7₂ birds.

Line 6₃ total RNA samples



Line 7₂ total RNA samples

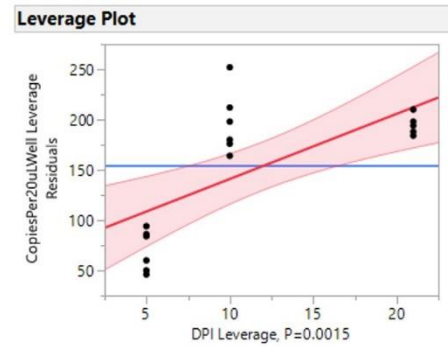


Figure S5. MDV miR-M12-3p microRNA expression leverage plots based on ddPCR absolute quantifications of the microRNA copies per 20- μ l reaction at 5, 10 and 21 days post MDV challenge for each individual sample with technical replicates. It is clearly shown that the miR-M12-3p microRNA expression of all birds at 10 and 21 dpi also differed from the expressions at 5 dpi in both line 6₃ and line 7₂ birds.

Supplementary Tables

Table S1. Numbers of reads aligned and mapped

Treatment group	Past filter reads	Clean reads	Mapped reads	Mapped rate (%)
Line6 ₃ 10 dpi Control	28,932,041	23,002,189	20,042,414	87.1
Line6 ₃ 10 dpi Challenged	27,792,916	22,147,027	19,434,748	87.8
Line6 ₃ 21 dpi Control	34,265,288	28,399,403	24,776,271	87.2
Line6 ₃ 21 dpi Challenged	26,638,242	21,678,806	18,826,655	86.8
Line7 ₂ 10 dpi Control	34,642,123	27,243,083	23,876,203	87.6
Line7 ₂ 10 dpi Challenged	32,238,388	25,455,920	22,635,032	88.9
Line7 ₂ 21 dpi Control	31,904,354	26,139,229	23,163,003	88.6
Line7 ₂ 21 dpi Challenged	29,734,617	24,212,812	20,912,014	86.4

Table S10. Primers used in ddPCR validation

Primer	Sequence (5'-3')	Validated in					
		MDV vs Control (L6 ₃)		MDV vs Control (L7 ₂)		L6 ₃ vs L7 ₂ (MDV)	
		10 DPI	21 DPI	10 DPI	21 DPI	10 DPI	21 DPI
ATP6V0D2	F: CAAGAATCCAGCAGAGAGAC	✓	✓	✓	✓		
	R: GTCTGCACTAGTCAAGATCC						
CLDN1	F: CAGGGAAGAGGTAGACAGAAAC			✓			
	R: CTGGAATGACTCAAGGAGAGAG						
F13A1	F: CCTACATCCCGATCCTCATAG			✓	✓	✓	✓
	R: GAATGGAGTTGTTCTCCCTG						
FABP3	F: CCCATAGCACCTTCAAGAAC			✓	✓		
	R: CATCTAGCTTGACCAAGGAC						
FN1	F: GAGTATACCTACAGCCTCACAG	✓					
	R: GCTTCTATCCCAGGAGACTATC						
LAMA1	F: CTCTGGTTCAGATAGTCTCCAG					✓	✓
	R: CCTGTAGCCTAATGCACATC						
LECT2	F: ATCTCTGGGTCAGGTTACTG			✓		✓	✓
	R: GGTATAGGTCTGAGGAGATGAG						
RGS5	F: GATCTACGAGGAGTTCATCCAG					✓	✓
	R: CCATCAGAGCAAAGATCCTC						
SERPINB10	F: CTCCTAGAGGGATCCAAAGTTC	✓					
	R: GAAAGGAGCAGTAGAGAGACAG						
STAR	F: CCTATCCTTTCCCCTAGTGAAG				✓		
	R: GACAAGTTTCACAGGGACTC						
TGM2	F: CTCTGCTGACTGAGTATGAGAC				✓		
	R: GATCTCTGGGTTCTGGATGTAG						
WNT7A	F: GAAAGTGGGTAGCAGAGAAG				✓		
	R: GGCGAATCCTATTCCGTATC						

Table S11. PCR primers used in ddPCR to examine expression of two MDV microRNAs associated with MDV latency

MDV microRNA	miRNA Sequence	Primer Sequence
miR-M3-3p	UGGGGGGUUCACAUUUU UAAGU	F: GCAGTGGGGGGTTCACATT
		R: AGCAGGTCCAGTTTTTTTTTTTTTTTACTTAA
miR-M12-3p	UGCAUAAUACGGAGGGU UCU	F: CGCGCAGTGCATAATACGGA
		R: GGTCCAGTTTTTTTTTTTTTTTAGAACCT