



Article

Identification and Validation of Ikaros (*IKZF1*) As a Cancer Driver Gene for Marek's Disease Virus-Induced Lymphomas

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Supplementary Materials:

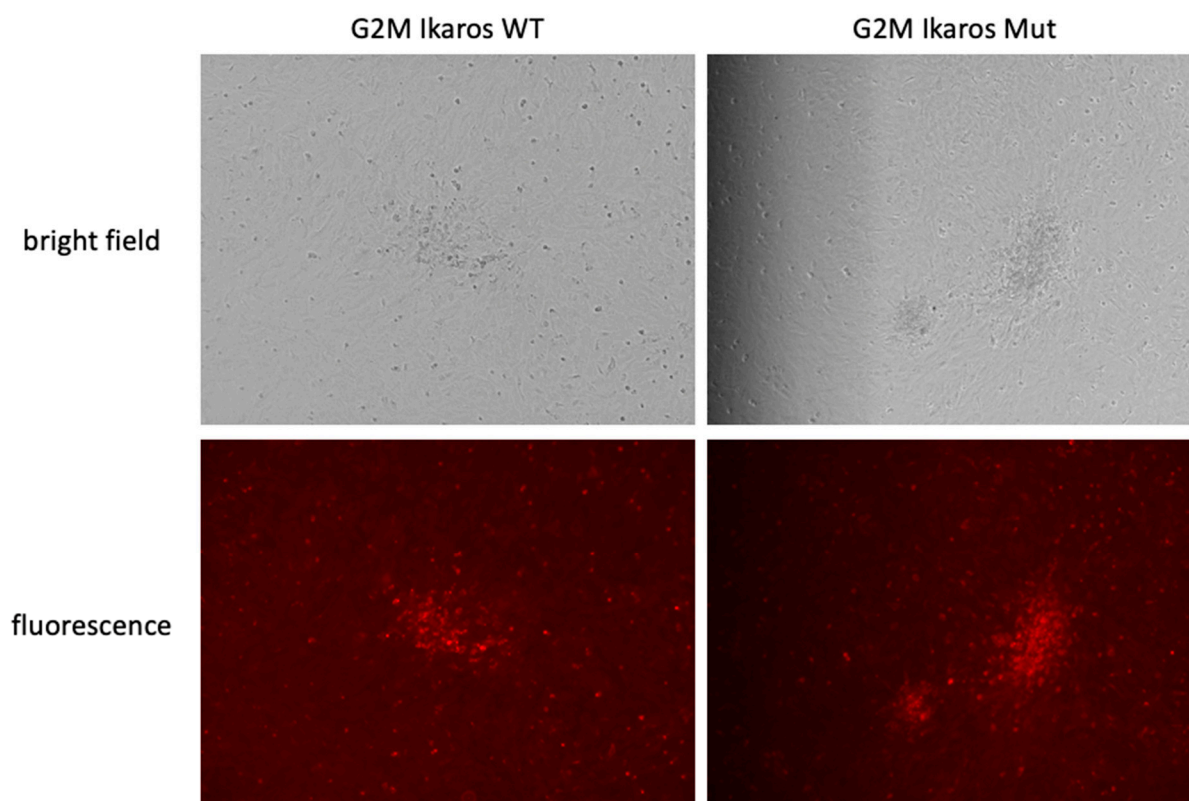


Figure S1. Bright field and immunofluorescent staining of G2M Ikaros WT and G2M Ikaros Mut to detect viral plaques and correspondence with expression of Ikaros-FLAG fusion using anti-FLAG tag antibody.

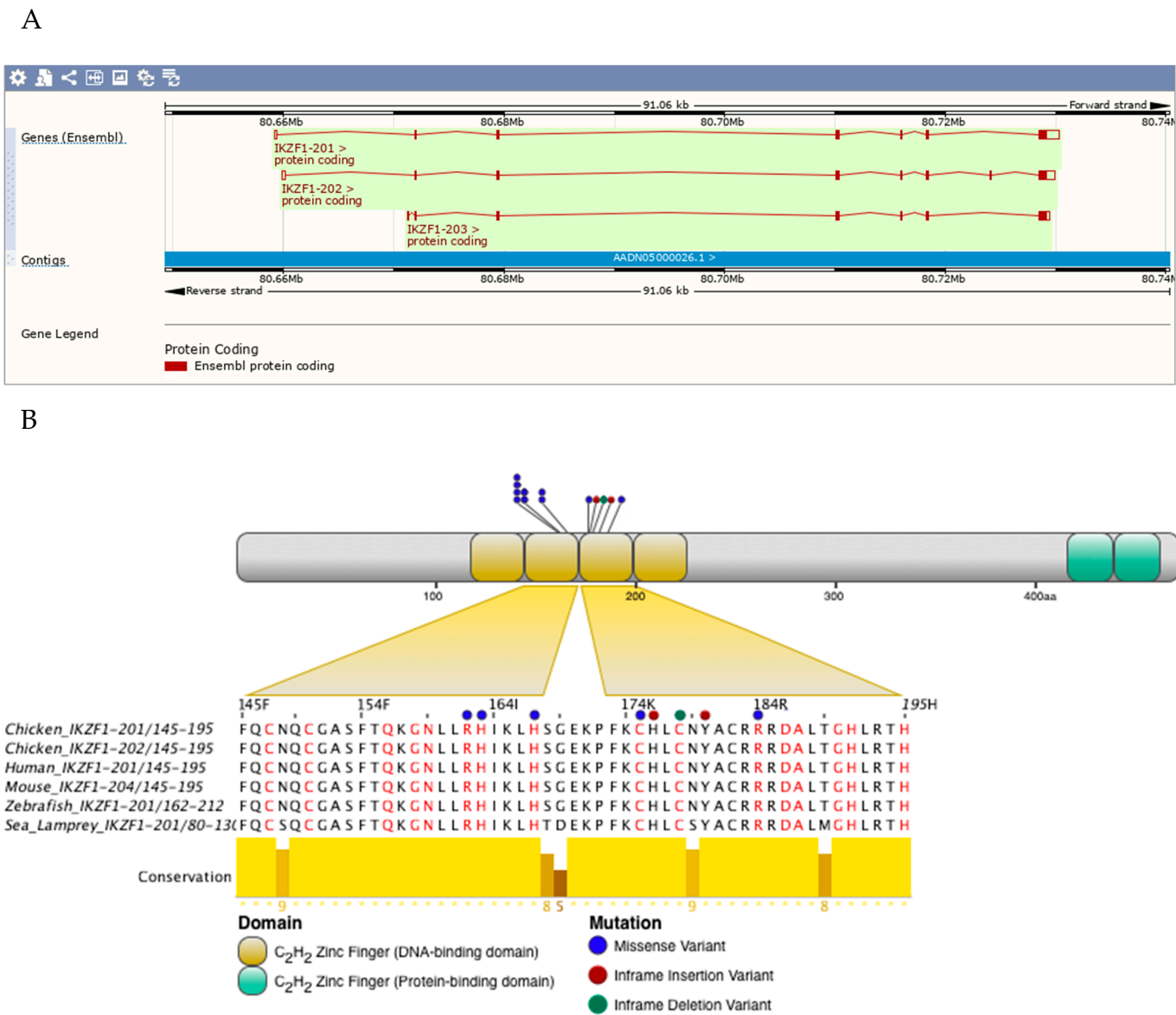


Figure S2. (A). Ensembl chicken IKZF1 gene information (ENSGALG00000013086) showing the orientation of the exons and the three known expressed isoforms. (B) The Ikaros protein with somatic non-synonymous mutations mapped on to the conserved amino acid sequences of DNA-binding zinc fingers 2 and 3. Amino acids essential for DNA-binding [66] are colored red, and conservation scores are represented as yellow bars plots under amino acid sequences. Somatic mutations are represented as colored dots (red, blue, and green).

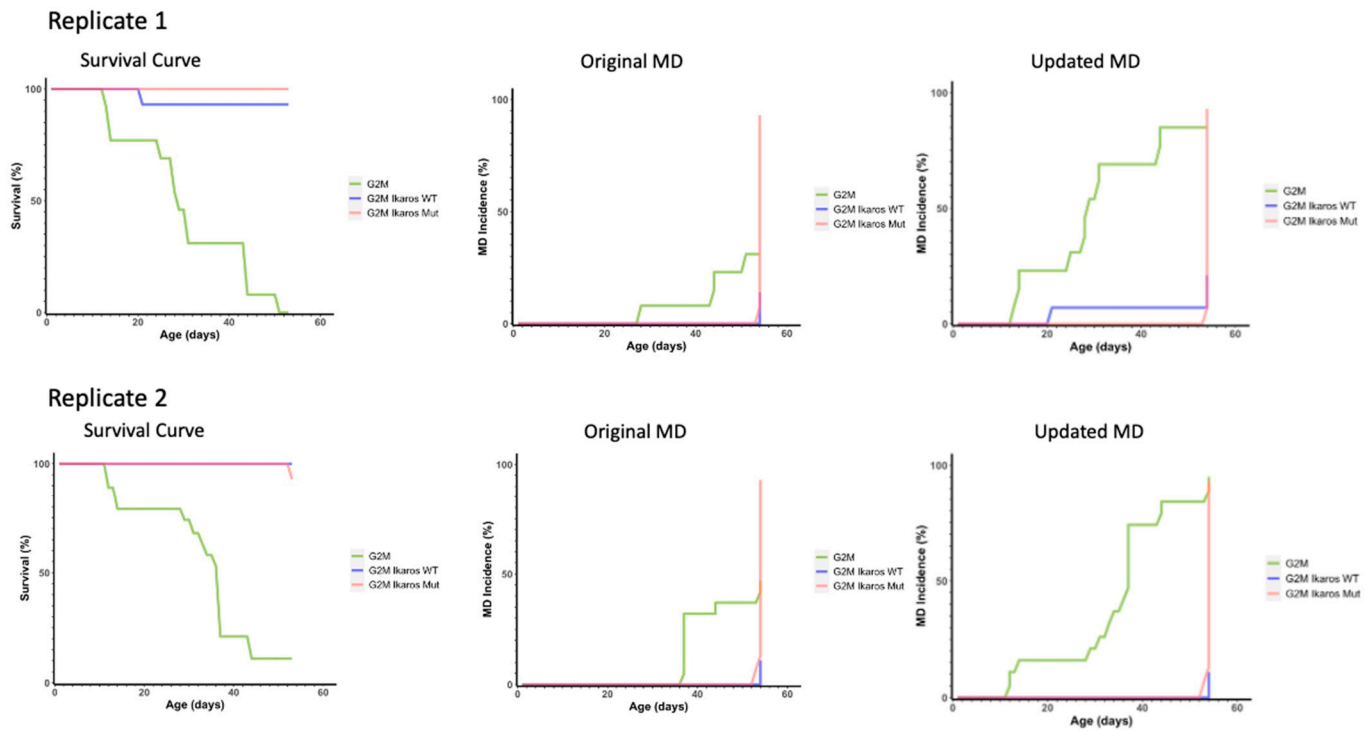


Figure S3. Comparison of survival and MD incidence over time for both replicates of bird experiment 2. The columns of the left give survival curves. The middle columns plot MD incidence over time using the traditional definition of MD that includes only birds with tumors or enlarged nerves at necropsy. The columns on the right show MD incidence over time that was expanded to include birds at least 7 days of age that died or developed clinical disease, and had bursa or thymic atrophy (BTA) with a score of 3 or higher (0 to 4 scale) at necropsy.

Table S1. MD incidence in comparing the viruses generated from the parental B40 BAC clone with viruses generated from G2M containing only a single repeat long (RL).

Replicate	Treatment	Total Birds	MD ¹	
			Count	Percent
1	none	5	0	0
	deltaRL G2M	16	16	100
	rMd5 B40	15	15	100
2	none	5	0	0
	deltaRL G2M	16	16	100
	rMd5 B40	17	16	94

¹MD is considered positive when a bird has enlarged nerves or evidence of a tumor.

Table S2. Distribution of tumors and nerve enlargements across organs for each recombinant MDV.

Replicate 1

Treatment	No. of birds	No. of birds with tumors	No. of tumors per bird (if present)	Organ with tumor						
				proventriculus	gonad	spleen	kidney	heart	liver	pancreas
none	6	0	0	0	0	0	0	0	0	0
G2M	13	2	3, 3	0	2	0	1	2	1	0
G2M Ikaros WT	14	2	1, 1	0	0	0	0	1	1	0
G2M Ikaros Mut	14	12	1, 2, 2, 2, 2, 3, 3, 3, 3, 3, 5	2	1	8	0	11	8	1

Treatment	No. of birds	No. of birds with nerve enlargement	Organ with nerve enlargement		
			vagus	brachial	sciatic
none	6	0	0	0	0
G2M	13	4	4	3	1
G2M Ikaros WT	14	1	0	0	1
G2M Ikaros Mut	14	6	6	2	1

Replicate 2

Treatment	No. of birds	No. of birds with tumors	No. of tumors per bird (if present)	Organ with tumor						
				proventriculus	gonad	spleen	kidney	heart	liver	pancreas
none	10	0	0	0	0	0	0	0	0	0
G2M	19	7	1, 1, 1, 1, 2, 2, 2	0	2	2	0	4	2	0
G2M Ikaros WT	19	2	1, 1	0	0	0	0	2	0	0
G2M Ikaros Mut	15	14	1, 1, 1, 2, 2, 2, 2, 2, 3, 3, 3, 3, 3	2	1	7	1	12	7	0

Treatment	No. of birds	No. of birds with nerve enlargement	Organ with nerve enlargement		
			vagus	brachial	sciatic
none	10	0	0	0	0
G2M	19	9	9	6	3
G2M Ikaros WT	19	0	0	0	0
G2M Ikaros Mut	15	3	3	3	2