Supplemental Material

Comorbid diabetes results in immune dysregulation and enhanced disease severity following MERS-CoV infection

Kirsten A. Kulcsar[1], Christopher M. Coleman[1#], Sarah E. Beck[2], Matthew B. Frieman[1*]

[1]University of Maryland School of Medicine, Department of Microbiology and Immunology, Baltimore MD USA 21201

[2]Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology, Baltimore, MD USA 21205

[#]Currently at University of Nottingham School of Life Sciences, Division of Infections, Immunity, and Microbes, Nottingham, UK

*To whom correspondence should be addressed: Matthew Frieman, 685 W. Baltimore St. Room 380, Baltimore, MD 21205; (410) 706-2539; mfrieman@som.umaryland.edu

Supplementary Figures and Tables

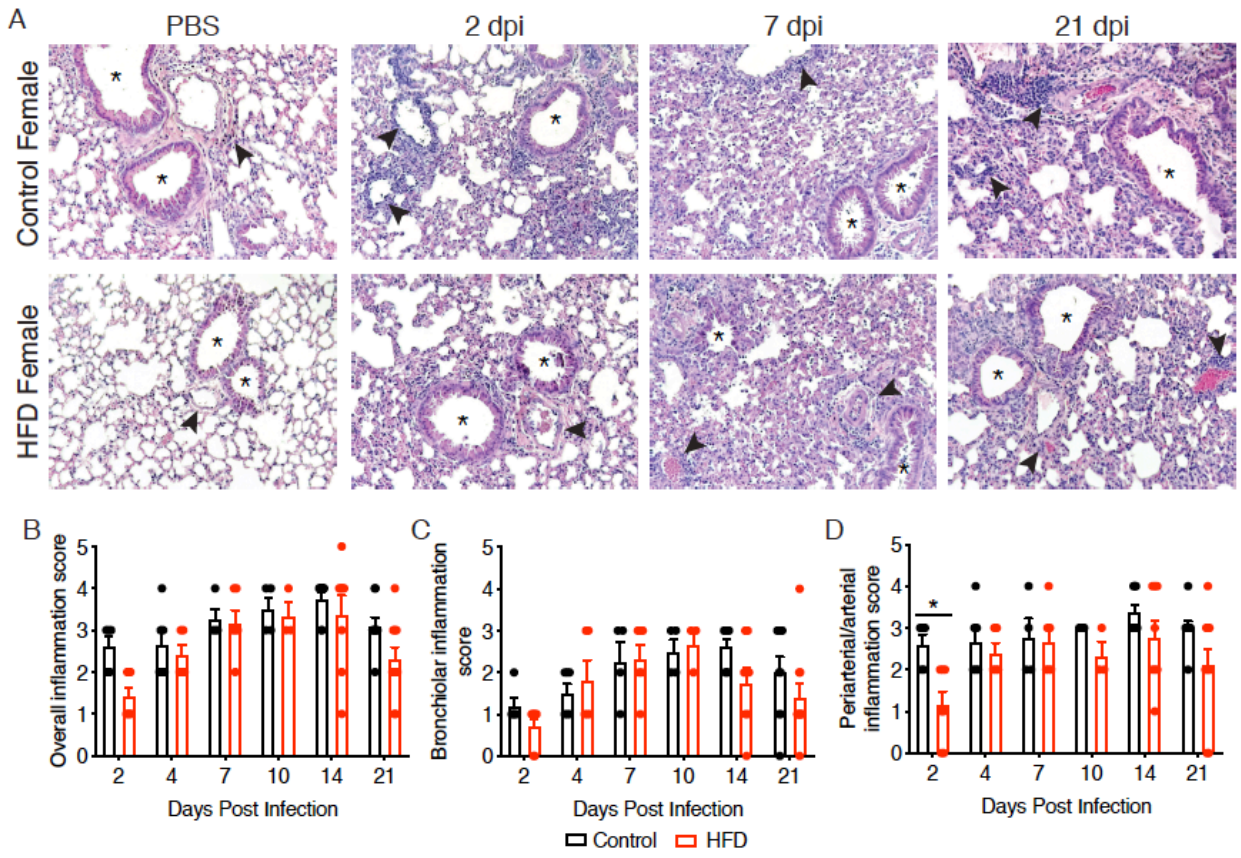

Figure S1. Lung histology shows a slight delay of inflammation in female HFD mice infected with MERS-CoV. Female HFD and control DPP4$^{H/M}$ mice were infected intranasally with 1.5x10^{5} pfu of MERS-CoV Jordan. A) Lungs were collected at days 2, 4, 7, 10, 14 and 21 after infection and fixed in 10% neutral buffered formalin for >24 hrs. Tissue was embedded in paraffin and 5um sections were cut and stained with hematoxylin and eosin. Blood vessels are marked by arrow heads and airways are marked by asterisk (*). Images are shown at 10x and are representative of n= 5-11 mice/group from 2-3 independent experiments. B) Overall lung inflammation, (C) bronchiolar inflammation, and (D) perivascular inflammation were scored by a board certified veterinarian. The data are pooled from 2-3 independent experiments, n= 5-11

mice/group. Data are presented as the mean and SEM. *p<0.05, ***p<0.001, and ****p<0.0001 as determined by two-way ANOVA with Sidak's post test.

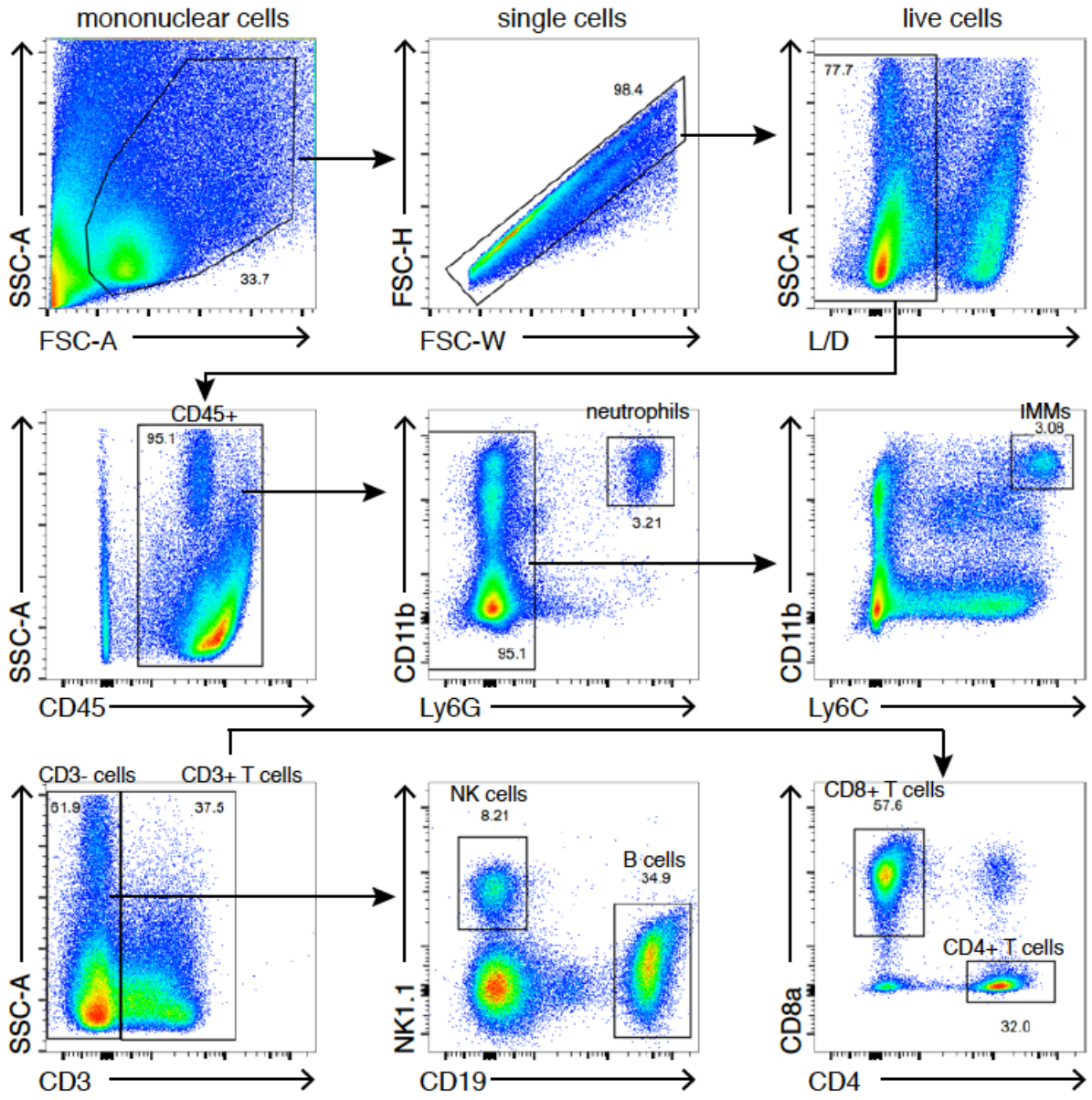

Figure S2. Flow cytometric gating scheme for phenotyping immune cells in the lungs of DPP4$^{H/M}$ infected with MERS-CoV. Diabetic and control male and HFD and control female DPP4$^{H/M}$ were infected intranasally with 1.5x10^{5} pfu of MERS-CoV Jordan. Lungs were collected at days 4, 7, 14, and 21 after infection or from uninfected mice and single cells were isolated from the tissue. Flow cytometric analysis was performed and analyzed to identify immune cell subsets in the lungs of uninfected and infected mice. Samples were gated on mononuclear cells, doublets were excluded, and live cells were identified. CD45+ cells were then

determined followed by subsequent subsetting into the various immune cell populations. CD3+ T cells (CD45+CD3+), CD4+ T cells (CD45+CD3+CD4+), CD8+ T cells (CD45+CD3+CD8+), B cells (CD45+CD3-CD19+), NK cells (CD45+CD3-NK1.1+), neutrophils (CD45+CD11b+Ly6G^{hi}), and inflammatory monocyte/macrophages (IMMs, CD45+Ly6G-CD11b+Ly6C^{hi}).

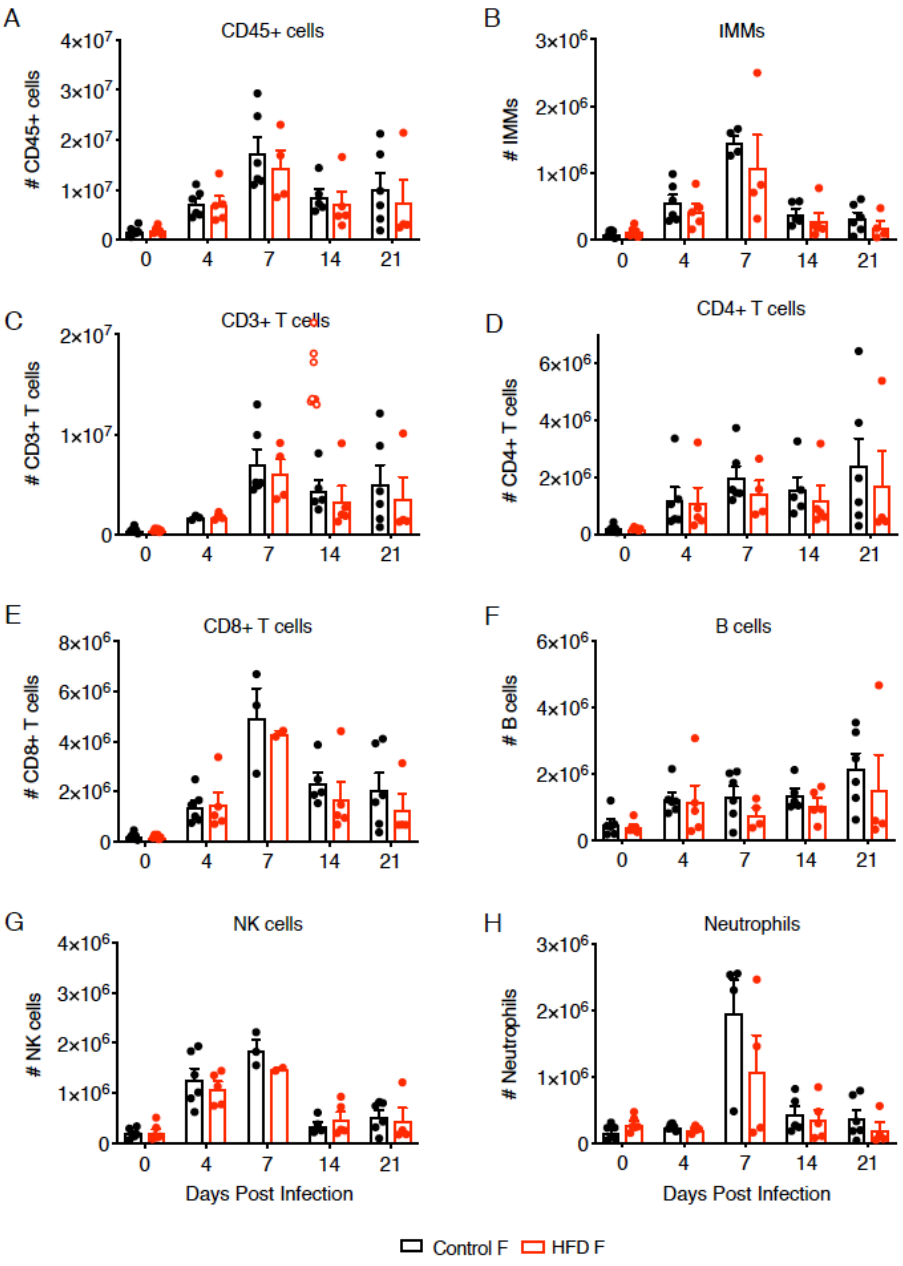

Figure S3. HFD female mice have few changes in immune cell infiltration following MERS-CoV infection. Female HFD and DPP4$^{H/M}$ were infected intranasally with 1.5x10^5 pfu of MERS-CoV Jordan. Lungs were collected at days 4, 7, 14, and 21 after infection or from uninfected mice and single cells were isolated from the tissue. Flow cytometric analysis was performed on the cells isolated from the lung. The number of (A) CD45+ cells, (B) inflammatory monocytes/macrophages, (C) CD3+ T cells, (D) CD4+ T cells, (E) CD8+ T cells, (F) B cells, (G)

natural killer (NK) cells, and (H) neutrophils was determined. The data are pooled from 2 independent experiments, n= 3-9 mice/group. Data are presented as the mean and SEM. *$p<0.05$ as determined by two-way ANOVA with Sidak's post test.

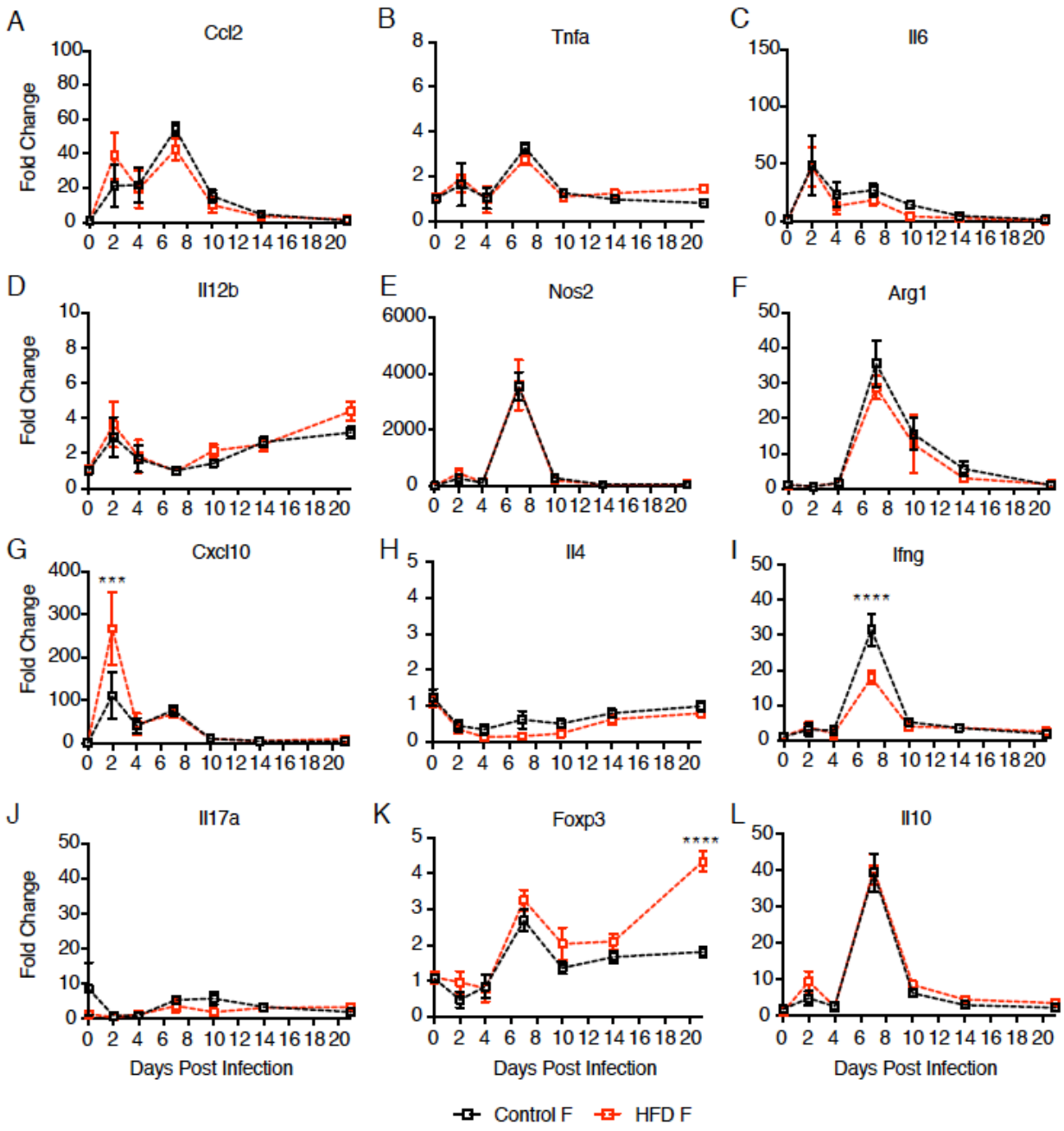

Figure S4. Cytokine and chemokine gene expression in the lungs of female HFD DPP4$^{H/M}$ mice following MERS-CoV infection. Female HFD and control DPP4$^{H/M}$ mice were infected intranasally with 1.5e5 pfu of MERS-CoV Jordan. Lung tissue was collected as days 2, 4, 7, 10, 14, and 21 after infection and from PBS infected mice and was homogenized in trizol. RNA was isolated, cDNA was synthesized, and gene expression was determined using qPCR. Gene

expression was normalized to GAPDH and fold change was calculated relative to PBS infected mice. Gene expression was determined for (A) Ccl2; (B) Tnfa; (C) Il6; (D) Il12b; (E) Nos2; (F) Arg1; (G) Cxcl10; (H) Il4; (I) Ifng; (J) Il17a; (K) Foxp3; and (L) Il10. Data are pooled from 2-3 independent experiments, n= 3-16 mice/group. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$ as determined by two-way ANOVA with Sidak's post test.

		Found dead		Euthanized		Total deaths	
		Control	**Diabetic/HFD**	**Control**	**Diabetic/HFD**	**Control**	**Diabetic/HFD**
Male	**# (%)**	2/14 (14%)	2/17 (12%)	3/14 (21%)	0/17 (0%)	5/14 (36%)	2/17 (12%)
	M.D.O.D.	10	8.5	11.7	n.a.	11	8.5
Female	**# (%)**	0/12 (0%)	0/14 (0%)	0/12 (0%)	0/14 (0%)	0/12 (0%)	0/14 (0%)
	M.D.O.D.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

*M.D.O.D. is mean day of death

Supplementary table 1. Mortality summary for DPP4$^{H/M}$ mice infected with a high dose of MERS-CoV.

Males (Control vs. Diabetic)											
CD45+ cells		#									
	DPI	Control	Diabetic	Adjusted p-value							
	0	4.30E+06	2.67E+06	0.9909							
	4	8.19E+06	6.79E+06	0.9946							
	7	1.90E+06	1.26E+07	0.1683							
	14	9.50E+06	9.41E+06	>0.9999							
	21	7.70E+06	6.61E+06	0.9974							
Infalmmatory monocyte/ macrophages (IMMs)		#			% of CD45+						
	DPI	Control	Diabetic	Adjusted p-value	Control	Diabetic	Adjusted p-value				
	0	3.69E+05	2.22E+05	0.9678	7.92	8.43	0.9987				
	4	7.55E+05	6.35E+05	0.9847	9.00	8.71	>0.9999				
	7	1.19E+06	5.13E+05	0.0122	6.21	4.32	0.6793				
	14	4.35E+05	5.39E+05	0.9890	4.73	5.44	0.9898				
	21	2.72E+05	2.56E+05	>0.9999	3.98	3.73	>0.9999				
Natural killer (NK) cells		#			% of CD45+						
	DPI	Control	Diabetic	Adjusted p-value	Control	Diabetic	Adjusted p-value				
	0	3.53E+05	2.48E+05	0.9972	8.63	9.60	0.9865				
	4	1.37E+06	1.55E+06	0.9689	16.42	21.66	0.0130				
	7	1.90E+06	1.83E+06	0.9999	8.66	11.20	0.7914				
	14	4.70E+05	5.37E+05	0.9995	5.26	5.52	>0.9999				
	21	4.19E+05	3.87E+05	>0.9999	5.13	7.05	0.6804				
Neutrophils		#			% of CD45+						
	DPI	Control	Diabetic	Adjusted p-value	Control	Diabetic	Adjusted p-value				
	0	7.12E+05	3.43E+05	0.4952	14.87	13.92	0.9952				
	4	2.58E+05	2.59E+05	>0.9999	3.17	4.14	0.9938				
	7	1.14E+06	9.09E+05	0.8741	5.54	6.86	0.9782				
	14	5.11E+05	3.35E+05	0.9308	5.67	3.42	0.7552				
	21	3.89E+05	2.69E+05	0.9865	5.36	3.98	0.9596				
B cells		#			% of CD45+						
	DPI	Control	Diabetic	Adjusted p-value	Control	Diabetic	Adjusted p-value				
	0	1.12E+06	6.07E+05	0.8689	26.28	21.63	0.3799				
	4	1.58E+06	9.49E+05	0.7089	19.73	14.69	0.2575				
	7	1.54E+06	8.05E+05	0.5254	8.06	6.23	0.9590				
	14	1.78E+06	1.19E+06	0.7101	19.47	12.97	0.0469				
	21	1.96E+06	1.19E+06	0.4046	25.86	18.91	0.0216				
CD3+ T cells		#			% of CD45+						
	DPI	Control	Diabetic	Adjusted p-value	Control	Diabetic	Adjusted p-value				
	0	8.79E+05	5.32E+05	0.9998	22.15	19.75	0.9408				
	4	2.64E+06	1.08E+06	0.9643	34.03	28.53	0.6824				
	7	7.15E+06	5.52E+06	0.7998	36.05	43.28	0.0669				
	14	4.14E+06	4.63E+06	0.9984	41.65	49.21	0.0366				
	21	3.11E+06	3.07E+06	>0.9999	40.98	43.88	0.7986				
CD4+ T cells		#			% of CD45+			% of CD3+			
	DPI	Control	Diabetic	Adjusted p-value	Control	Diabetic	Adjusted p-value	Control	Diabetic	Adjusted p-value	
	0	4.03E+05	2.44E+05	0.9991	10.12	8.87	0.9858	46.8	47.7	0.9999	
	4	1.14E+06	5.54E+05	0.7289	14.07	8.03	0.0302	25.27	26.37	0.9997	
	7	2.25E+06	1.10E+06	0.0854	12.33	9.10	0.4802	35.65	21.06	<0.0001	
	14	1.39E+06	1.35E+06	>0.9999	13.60	14.17	0.9994	32.96	28.87	0.5595	
	21	1.22E+06	1.23E+06	>0.9999	15.70	15.86	>0.9999	38.43	35.69	0.8519	
CD8+ T cells		#			% of CD45+			% of CD3+			
	DPI	Control	Diabetic	Adjusted p-value	Control	Diabetic	Adjusted p-value	Control	Diabetic	Adjusted p-value	
	0	3.88E+05	2.30E+05	0.9999	9.73	8.58	0.9714	44.1	42.77	0.9986	
	4	1.43E+06	1.06E+06	0.9902	17.53	14.67	0.3891	54.77	45.57	0.1422	
	7	5.47E+06	4.41E+06	0.8194	21.97	25.73	0.4373	52.53	58.88	0.4278	
	14	2.29E+06	2.64E+06	0.9912	23.65	28.34	0.0200	56.54	57.63	0.9965	
	21	1.51E+06	1.36E+06	0.9998	20.14	20.56	0.9995	49	47.3	0.9693	

Supplementary table 2. Summary of flow cytometry statistics for immune cell populations in the lungs of male diabetic and control DPP4$^{H/M}$ mice during MERS-CoV infection.

Female (Control vs. HFD)											
CD45+ cells		#									
	DPI	Control	HFD	Adjusted p-value							
	0	1.81E+06	1.94E+06	>0.9999							
	4	7.25E+06	7.15E+06	>0.9999							
	7	1.74E+07	1.44E+07	0.9175							
	14	8.63E+06	7.18E+06	0.9963							
	21	1.02E+07	7.51E+06	0.9468							
Infalmmatory monocyte/ macrophages (IMMs)		#			% of CD45+						
	DPI	Control	HFD	Adjusted p-value	Control	HFD	Adjusted p-value				
	0	9.93E+04	1.25E+05	>0.9999	5.53	6.27	0.9722				
	4	5.58E+05	4.37E+05	0.9807	7.58	5.90	0.5786				
	7	1.46E+06	1.09E+06	0.4613	8.18	6.87	0.8775				
	14	3.94E+05	2.85E+05	0.9901	4.62	3.66	0.9421				
	21	3.26E+05	1.97E+05	0.9801	3.38	3.28	>0.9999				
Natural killer (NK) cells		#			% of CD45+						
	DPI	Control	HFD	Adjusted p-value	Control	HFD	Adjusted p-value				
	0	2.00E+05	2.16E+05	>0.9999	11.99	10.53	0.9644				
	4	1.27E+06	1.09E+06	0.8783	17.40	16.60	0.9981				
	7	1.86E+06	1.47E+06	0.6565	8.30	7.56	0.9998				
	14	3.55E+05	4.74E+05	0.9842	4.10	6.80	0.7512				
	21	5.34E+05	4.55E+05	0.9978	5.88	6.69	0.9985				
Neutrophils		#			% of CD45+						
	DPI	Control	HFD	Adjusted p-value	Control	HFD	Adjusted p-value				
	0	1.73E+05	2.97E+05	0.9930	9.24	15.83	0.0003				
	4	2.50E+05	2.17E+05	>0.9999	3.91	3.38	0.9988				
	7	1.97E+06	1.08E+06	0.0359	9.48	6.00	0.2795				
	14	4.35E+05	3.68E+05	0.9998	4.90	4.83	>0.9999				
	21	3.85E+05	2.10E+05	0.9806	3.60	3.04	0.9987				
B cells		#			% of CD45+						
	DPI	Control	HFD	Adjusted p-value	Control	HFD	Adjusted p-value				
	0	4.91E+05	4.08E+05	>0.9999	26.27	21.03	0.3205				
	4	1.25E+06	1.16E+06	>0.9999	17.70	13.63	0.6346				
	7	1.35E+06	7.84E+05	0.8482	7.78	5.33	0.9492				
	14	1.36E+06	1.07E+06	0.9883	16.26	16.80	>0.9999				
	21	2.15E+06	1.53E+06	0.7820	25.05	17.88	0.1418				
CD3+ T cells		#			% of CD45+						
	DPI	Control	HFD	Adjusted p-value	Control	HFD	Adjusted p-value				
	0	4.71E+05	4.45E+05	>0.9999	25.35	23.37	0.9502				
	4	1.70E+06	1.81E+06	>0.9999	34.20	36.00	0.9929				
	7	7.12E+06	6.12E+06	0.9879	40.58	42.70	0.9589				
	14	4.41E+06	3.41E+06	0.9866	49.94	44.12	0.2199				
	21	5.12E+06	3.56E+06	0.9201	45.77	47.90	0.9576				
CD4+ T cells		#			% of CD45+			% of CD3+			
	DPI	Control	HFD	Adjusted p-value	Control	HFD	Adjusted p-value	Control	HFD	Adjusted p-value	
	0	2.05E+05	1.92E+05	>0.9999	11.10	10.41	0.9996				
	4	1.19E+06	1.10E+06	>0.9999	14.47	12.82	0.9804	29.57	25.30	0.7876	
	7	1.98E+06	1.42E+06	0.9737	11.35	9.35	0.9660	28.07	22.08	0.2947	
	14	1.56E+06	1.19E+06	0.9951	17.12	15.68	0.9912	33.82	35.46	0.9792	
	21	2.39E+06	1.71E+06	0.9365	20.10	19.08	0.9984	43.35	39.90	0.7738	
CD8+ T cells		#			% of CD45+			% of CD3+			
	DPI	Control	HFD	Adjusted p-value	Control	HFD	Adjusted p-value	Control	HFD	Adjusted p-value	
	0	2.17E+05	1.83E+05	>0.9999	11.48	9.46	0.7265				
	4	1.37E+06	1.46E+06	>0.9999	18.45	19.16	0.9968	49.15	47.30	0.9790	
	7	4.95E+06	4.31E+06	0.9763	20.83	22.15	0.9917	52.80	51.20	0.9954	
	14	2.34E+06	1.71E+06	0.8983	27.22	22.58	0.0689	54.92	51.38	0.7489	
	21	2.08E+06	1.28E+06	0.7818	19.93	21.25	0.9616	44.12	44.25	>0.9999	

Supplementary table 3. Summary of flow cytometry statistics for immune cell populations in the lungs of female HFD and control DPP4$^{H/M}$ mice during MERS-CoV infection.