Interleukin 6 Regulates Psoriasiform Inflammation Associated Thrombosis

Yunmei Wang[1†], Jackelyn B. Golden[2†], Yi Fritz[2†], Xiufen Zhang[2], Doina Diaconu[2], Maya I. Camhi[2], Huiyun Gao[1], Sean M. Dawes[2], Xianying Xing[3], Santhi K. Ganesh[4], Johann E. Gudjonsson[3], Daniel I. Simon[1], Thomas S. McCormick[2], Nicole L. Ward[2*]

[1]Cardiovascular Research Institute, Department of Medicine, Case Western Reserve University, Cleveland, OH.

[2]Department of Dermatology, Case Western Reserve University, Cleveland, OH.

[3]Department of Dermatology, University of Michigan, Ann Arbor, MI.

[4] Department of Internal Medicine, Division of Cardiovascular Medicine, and Department of Human Genetics, Univ. of Michigan, Ann Arbor, MI.

[†]Contributed equally to this work

[*]Address for correspondence:

Dr. Nicole L. Ward
Case Western Reserve University
Department of Dermatology
BRB519, 10900 Euclid Ave,
Cleveland, OH 44106
216-368-1111 office phone
216-368-0212 fax
nlw4@cwru.edu

Supplemental Figure 1. Characterization of KC-Tie2x*Mrp14*$^{-/-}$ mouse skin. (A). Transcript and (B). protein expression of Tie2 and MRP14 in control (n=5-9), KC-Tie2 (n=9), *Mrp14*$^{-/-}$ (n=11-13) and KC-Tie2x*Mrp14*$^{-/-}$ mice (n=11). P values are as indicated. (C). Immunohistochemistry staining of immune cells in control, KC-Tie2, *Mrp14*$^{-/-}$ and KC-Tie2x*Mrp14*$^{-/-}$ mice. Left panels: Representative immunohistochemistry of dorsal skin sections stained for CD11c, F4/80, CD4 and CD8 and the cell proliferation marker, Ki67.

Supplemental Figure 2. KC-Tie2x*IL-6*$^{-/-}$ mice have sustained skin inflammation. Quantification of (A). CD4, (B). CD8, (C). CD11b and D). F4/80 positively stained immune cells in dorsal skin of control (n=4), KC-Tie2 (n=7), *IL-6*$^{-/-}$ (n=6) and KC-Tie2x*IL-6*$^{-/-}$(n=8) mice. P values are as indicated.

Supplemental Figure 3. IL-6 deficiency reverses shortened thrombus occlusion time in the K5-IL-17C psoriasis mouse model. Occlusion times (mean ± SEM, mins) following rose bengal elicited photochemical injury of the carotid artery in control (n=9), K5-IL-17C (n=5), *IL-6*$^{-/-}$ (n=8), and K5-IL-17Cx*IL-6*$^{-/-}$ (n=15) mice. Each dot represents one individual mouse. P values are as indicated.

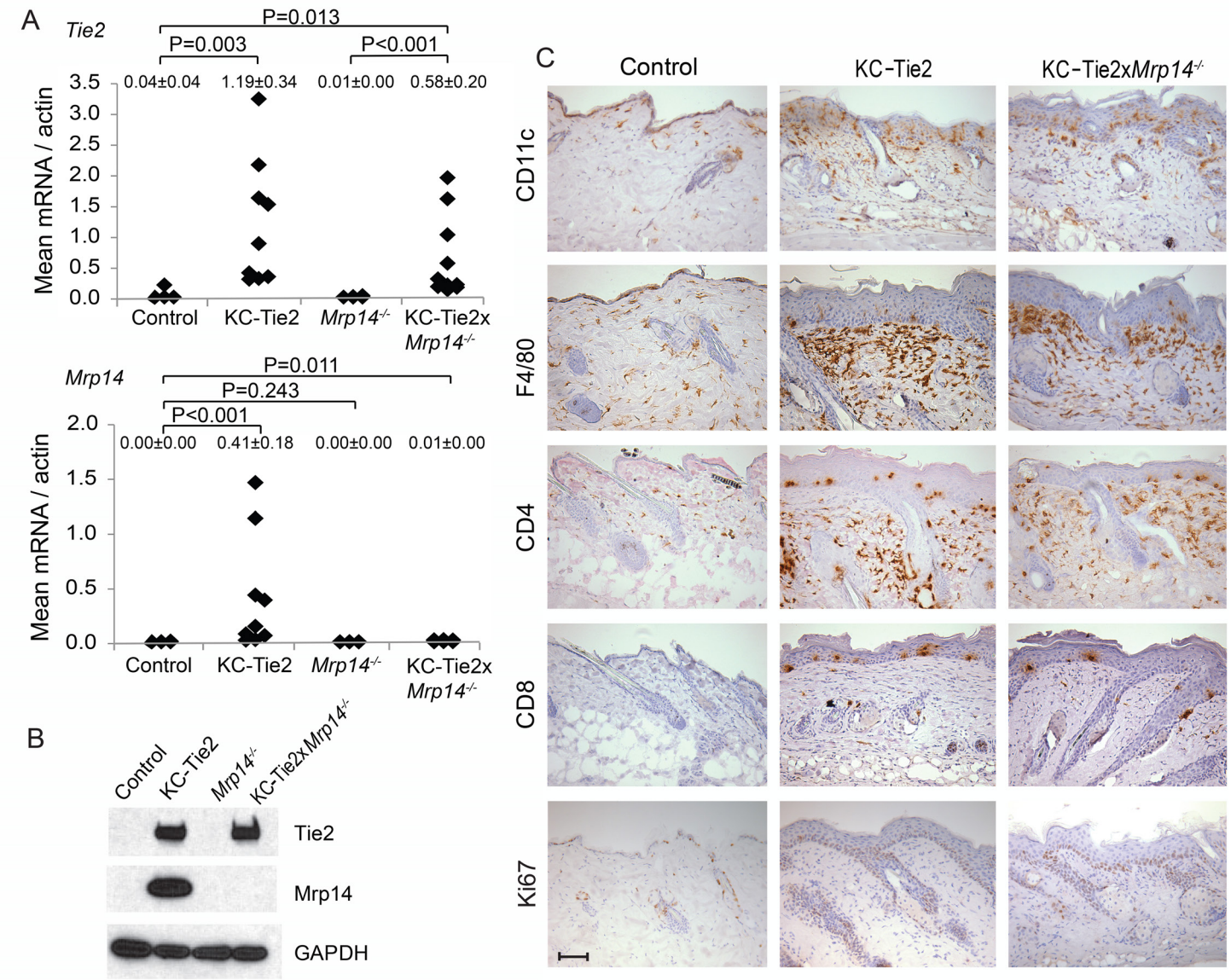
Supplemental Figure 4. Gating strategy used for flow cytometry. (A). Monocytes were selected based on FSC-A versus SSC-A. (B). Live cells were then selected using FV450 versus FSC-H and (C). single cells were gated based on FSC-A versus FSC-H. (D). Neutrophils were defined as CD11b^{+} and Ly6G^{+}. CD11b^{+}Ly6G^{neg} cells were taken into (E). where CD11b^{+}Ly6C^{high} events were defined as monocytes. Gates in D and E were determined by isotypes of CD11b, Ly6G, and Ly6C.

Supplemental Table 1. Average immune cell counts/field of view ± SEM in dorsal skin of mice.

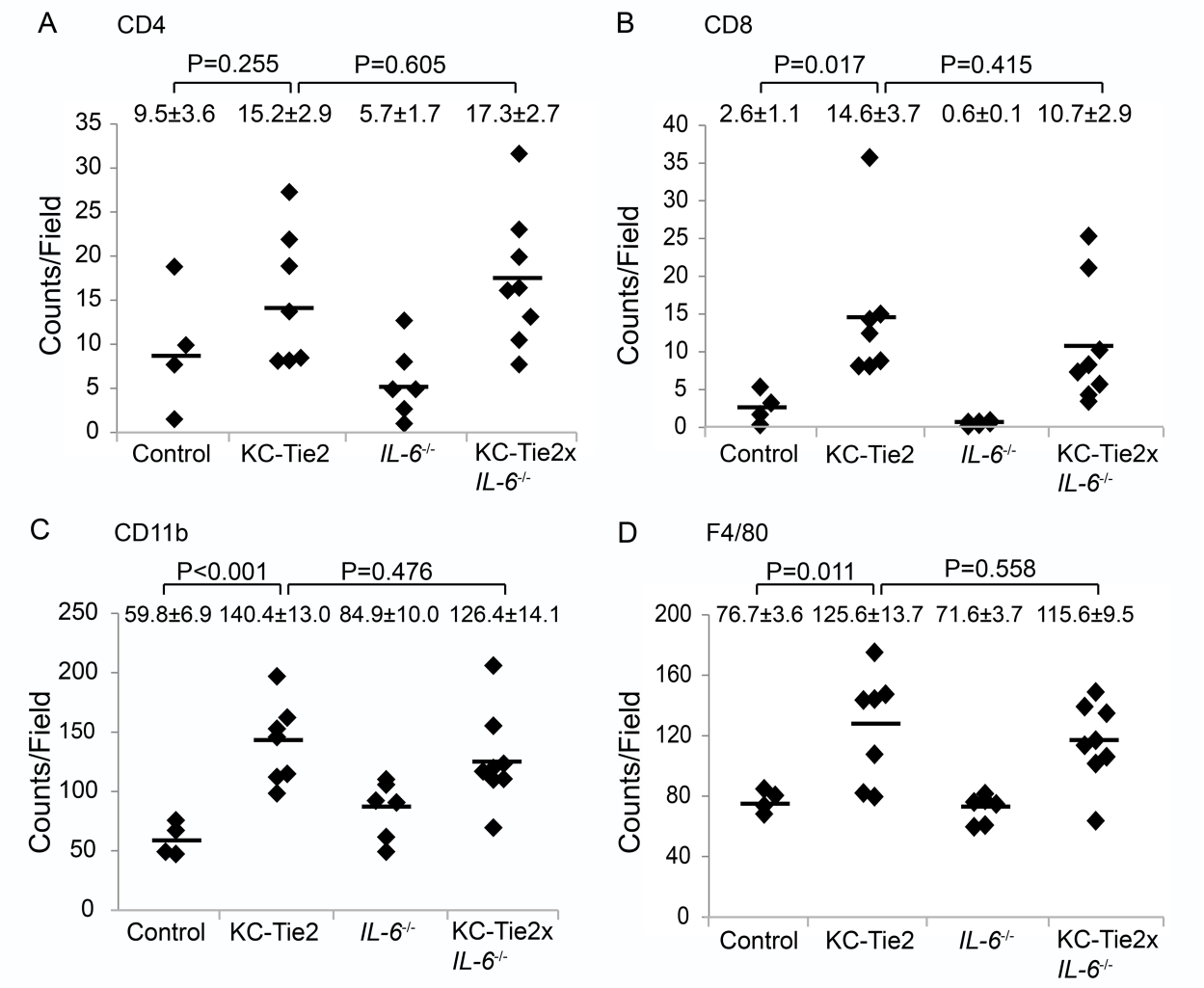
	CD11c	**F4/80**	**CD4**	**CD8**
Control	31.06 ± 6.36	41.49 ± 6.00	5.74 ± 2.14	0.60 ± 0.18
***Mrp14*$^{-/-}$**	24.11 ± 2.98	44.00 ± 7.55	3.79 ± 0.68	1.04 ± 0.29
KC-Tie2	61.10 ± 7.85*	95.95 ± 9.38*	19.71 ± 3.75*	10.46 ± 1.35*
KC-Tie2x*Mrp14*$^{-/-}$	36.36 ± 7.54^{+}	79.32 ± 7.55*	17.36 ± 1.05*	8.90 ±1.88*

* P<0.05 vs. control; + P<0.05 vs. KC-Tie2; Student's T-test.

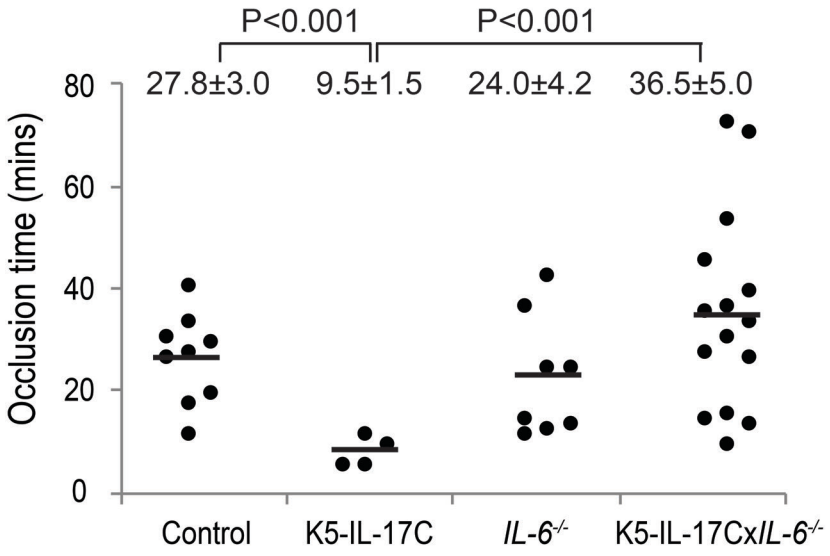
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4