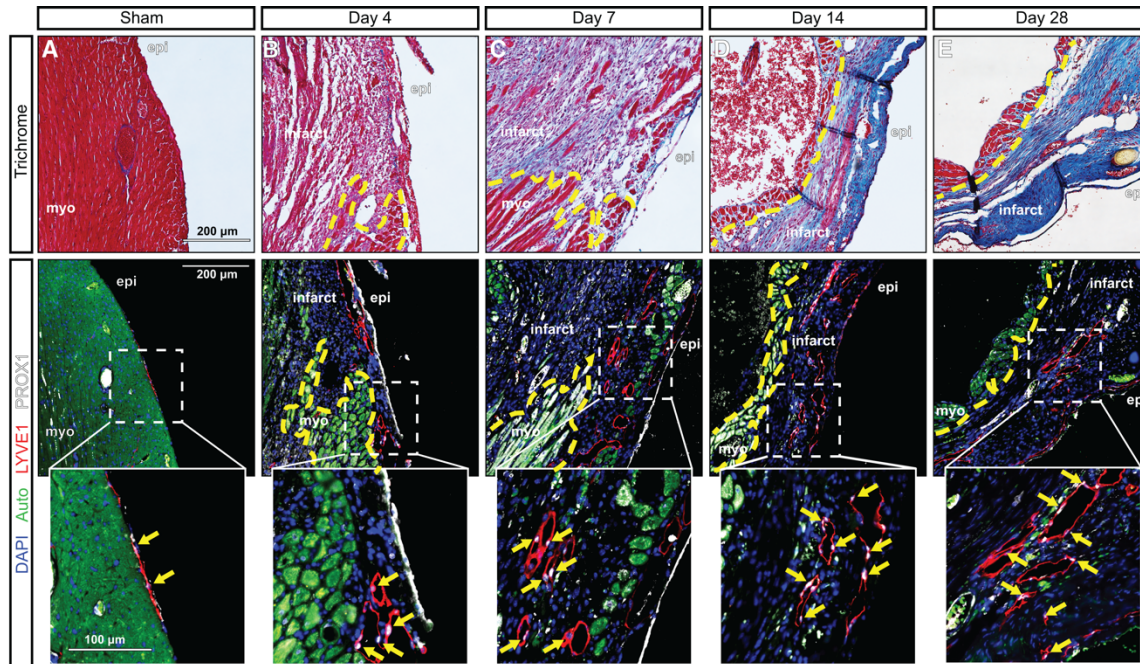


SUPPLEMENTAL MATERIAL for

“Genetic blockade of lymphangiogenesis does not impair cardiac function after myocardial infarction”

TCS Keller IV, L Lim ...ML Kahn *JCI* (2021)

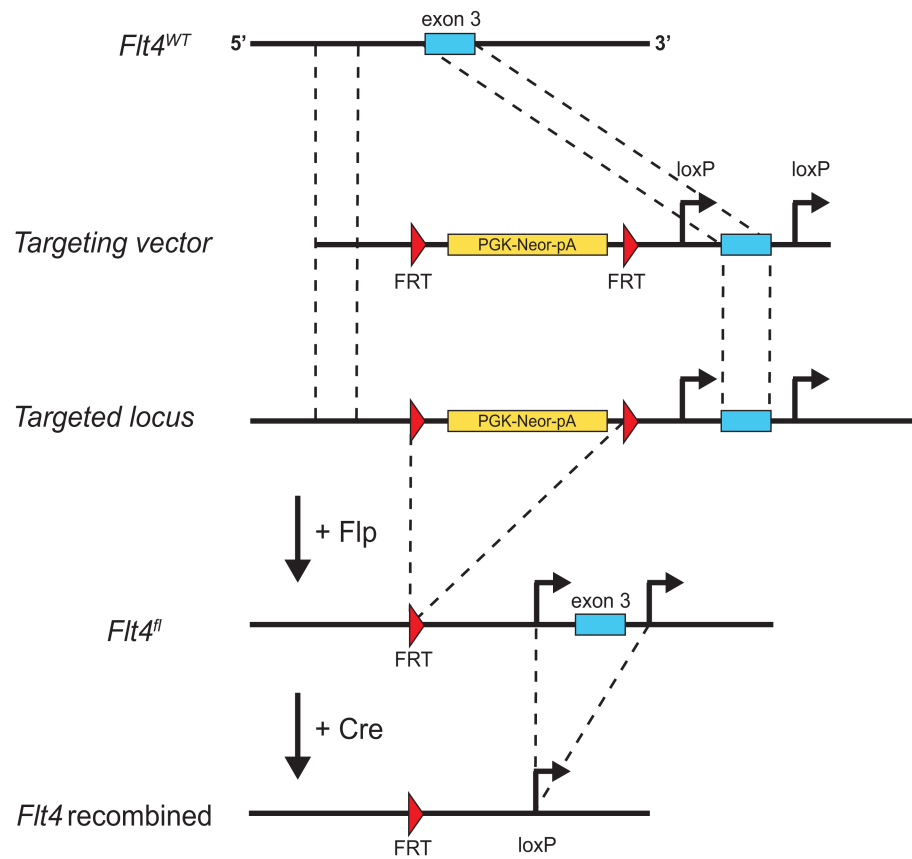
Supplemental Figure 1



Supplementary Figure 1: Growth of lymphatic vessels in the infarct zone after MI.

(A) A sham operated wild-type heart was stained using Masson's Trichrome and antibodies to detect LYVE1 and PROX1 (lower panels). (B-E) Adjacent sections from the same infarct zone in wild-type animal were stained using Masson's Trichrome stain (B-E, top) and immunostaining for the lymphatic endothelial markers LYVE1 and PROX1 (B-E, lower panels) at 4 (B), 7 (C), 14 (D), and 28 (E) days after MI. Yellow dotted lines indicate border of viable myocardium (highly autofluorescent, green) and infarcted myocardium. "Epi" denotes epicardium; "myo" denotes viable myocardium; "infarct" denotes infarcted myocardium. Yellow arrows indicate LYVE1+;PROX1+ lymphatic endothelial cells. Images are representative of >3 animals at each time point.

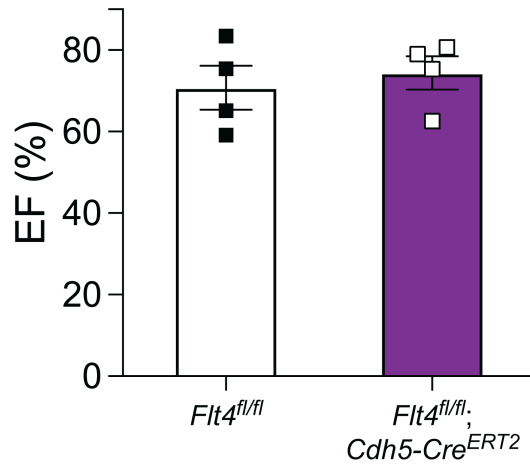
Supplemental Figure 2



Gene targeting strategy to generate a conditional *Flt4^{fl/fl}* allele. The targeting vector shown was used to replace exon 3 of the *Flt4* allele with a floxed exon 3 preceded by a FRT-flanked Neomycin resistance cassette. *Flp*-mediated recombination was used to excise the Neomycin-resistance cassette and generate the loxP-flanked exon 3 used in these studies.

Supplemental Figure 3

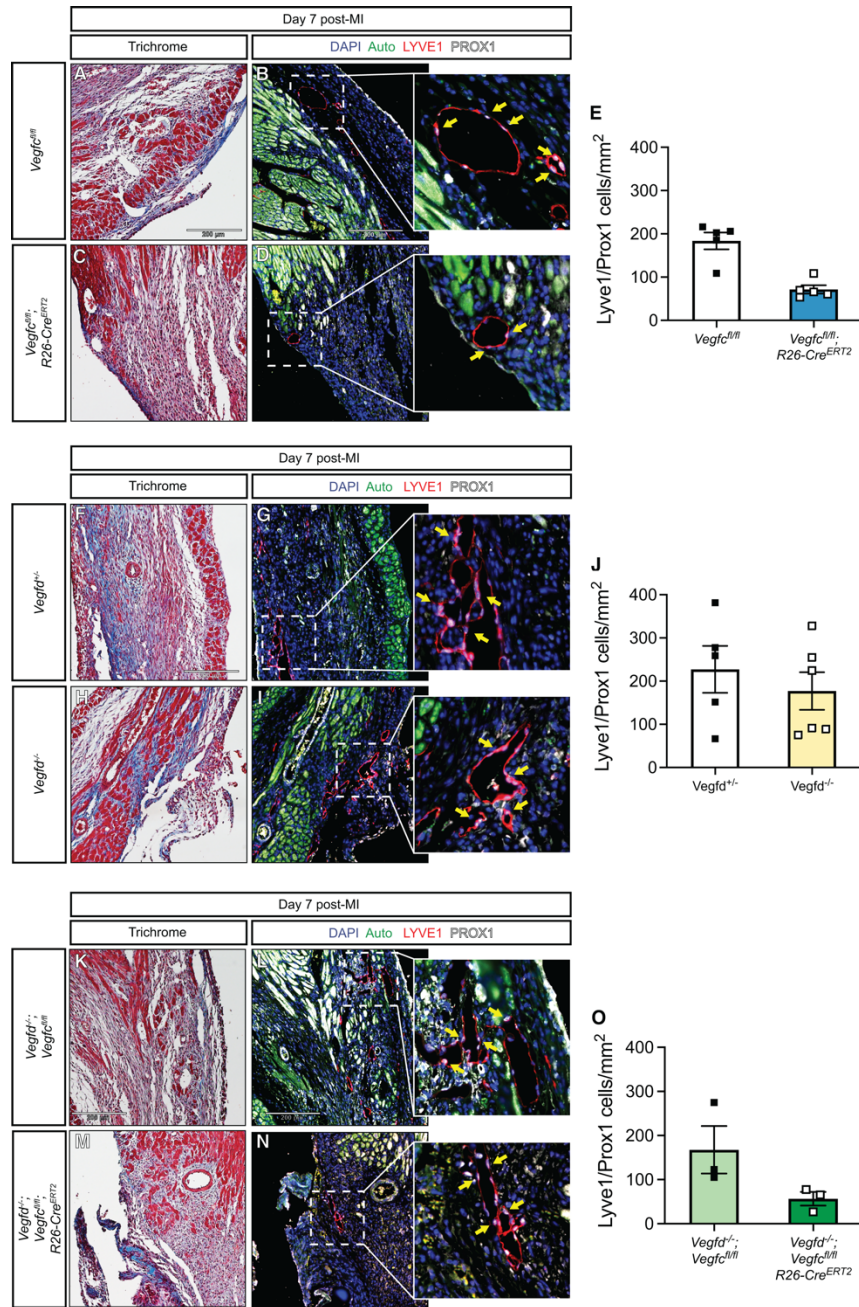
Baseline Ejection Fraction



Baseline ejection fraction of animals after deletion of *Flt4* using the *Cdh5-Cre^{ERT2}*

driver. Ejection fraction was assessed prior to myocardial infarction of animals after *Flt4* deletion by tamoxifen gavage (n = 4, 4). Tamoxifen was allowed to wash out for 14 days before cardiac functional assessment. Statistical comparison was made with a two-tailed t-test.

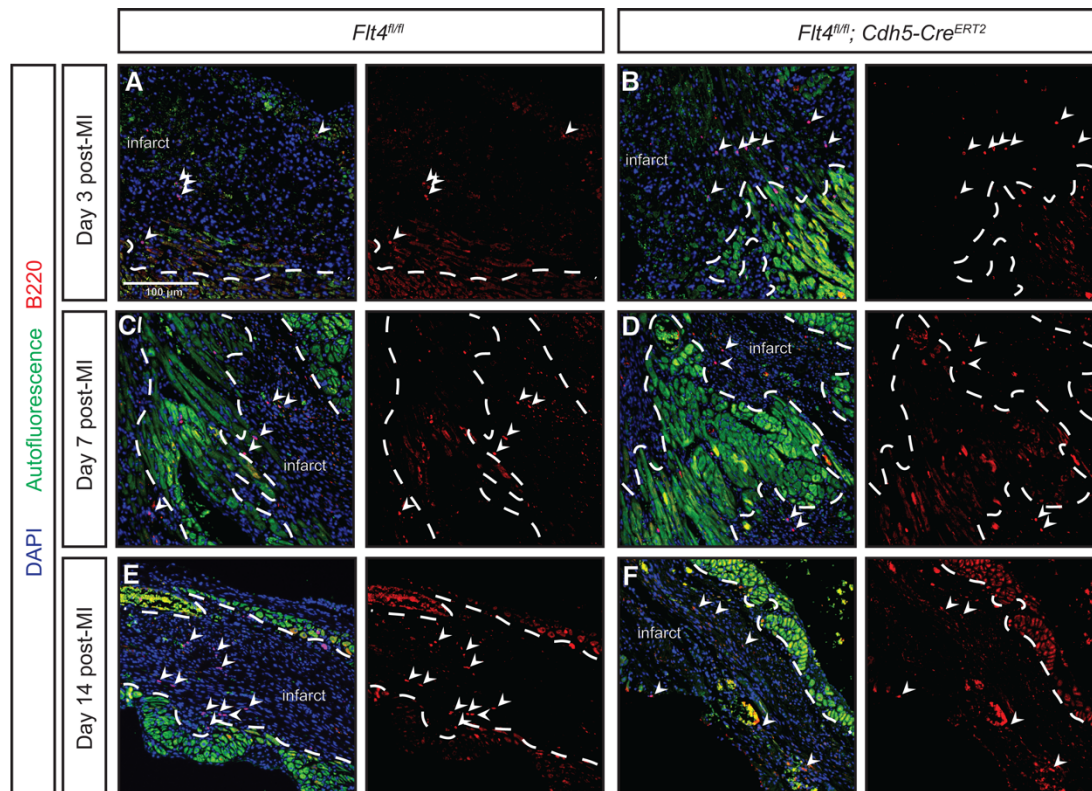
Supplemental Figure 4



Characterization of infarct lymphangiogenesis in mice lacking VEGF-C only, VEGF-D only, or both VEGF-C and VEGF-D. (A-D) Infarct lymphangiogenesis in mice lacking VEGF-C 7 days after MI. (A, C) Masson's Trichrome stain of the infarct zones of

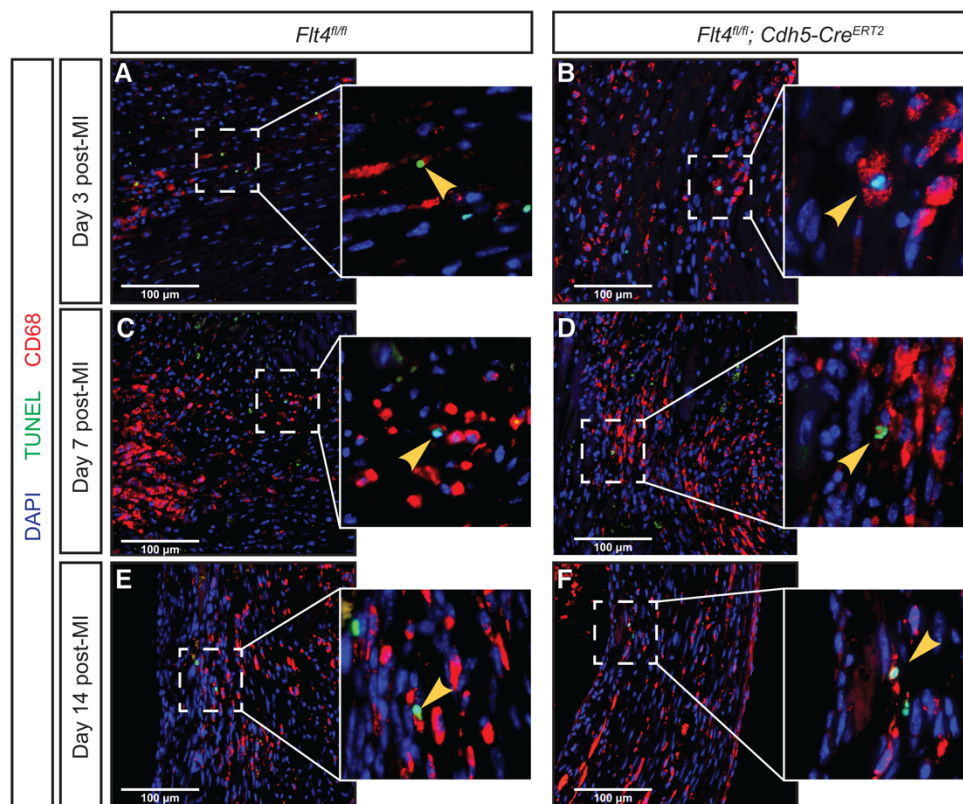
Vegfc^{fl/fl} and *Vegfc^{fl/fl}; R26-Cre^{ERT2}* hearts. **(B, D)** Immunostaining for LYVE1 and PROX1 in the infarct zones of *Vegfc^{fl/fl}* and *Vegfc^{fl/fl}; R26-Cre^{ERT2}* hearts. Inset images show the boxed regions in **B** and **D** at higher magnification. **(E)** The number of LYVE1+/PROX1+ lymphatic endothelial cells was measured per mm² in the infarct zone of the indicated animals (n = 5, 5). **(F-I)** Infarct lymphangiogenesis in mice lacking VEGF-D 7 days after MI. **(F, H)** Masson's Trichrome stain of the infarct zones of *Vegfd^{+/-}* and *Vegfd^{-/-}* hearts. **(G, I)** Immunostaining for LYVE1 and PROX1 in the infarct zones of *Vegfd^{+/-}* and *Vegfd^{-/-}* hearts. Inset images show the boxed regions in **G** and **I** at higher magnification. **(J)** The number of LYVE1+/PROX1+ lymphatic endothelial cells was measured per mm² in the infarct zone of the indicated animals (n = 5, 6). **(K-N)** Infarct lymphangiogenesis in mice lacking VEGF-C and VEGF-D 7 days after MI. **(K, M)** Masson's Trichrome stain of the infarct zones of *Vegfd^{-/-}; Vegfc^{fl/fl}* control and *Vegfd^{-/-}; Vegfc^{fl/fl}; R26-Cre^{ERT2}* hearts. **(L, N)** Immunostaining for LYVE1 and PROX1 in the infarct zones of *Vegfd^{-/-}; Vegfc^{fl/fl}* control and *Vegfd^{-/-}; Vegfc^{fl/fl}; R26-Cre^{ERT2}* hearts. Inset images show the boxed regions in **L** and **N** at higher magnification. **(O)** The number of LYVE1+/PROX1+ lymphatic endothelial cells was measured per mm² in the infarct zone of the indicated animals (n = 3, 3). Bar graphs are centered on mean, error bars represent SEM. Statistical comparison was made with a two-tailed t-test.

Supplemental Figure 5



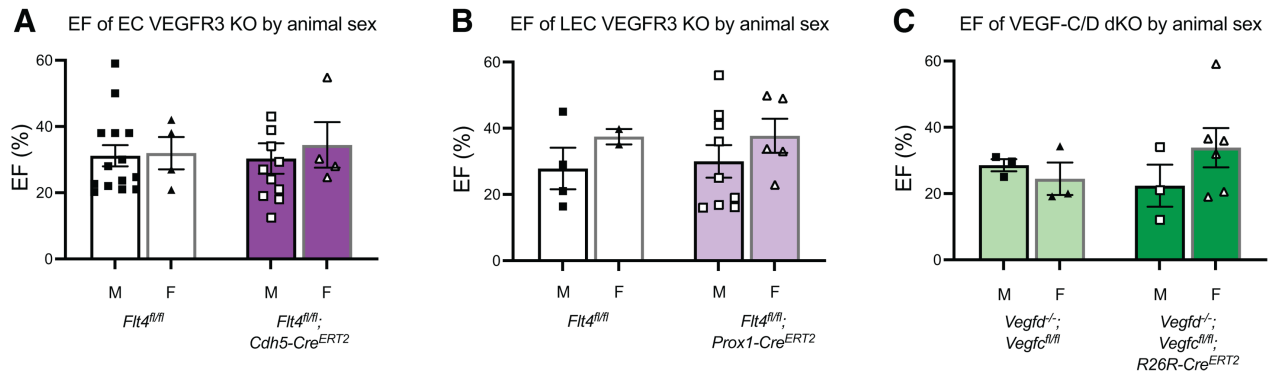
Assessment of the immune cell populations in the infarcted heart revealed low incidence of B cell lymphocytes. B cells, marked by high B220 expression (red), were examined histologically at 3 (**A, B**), 7 (**C, D**), and 14 (**E, F**) days post-MI in *Flt4^{fl/fl}* (**A, C, E**) and *Flt4^{fl/fl}; Cdh5-Cre^{ERT2}* (**B, D, F**) animals. Individual cells within the infarct zone are marked by arrowheads. The infarct zone is determined by low tissue autofluorescence (green), is outlined by dashed white line, and labeled “infarct”. Images are representative of >4 animals assayed at each time point and genotype.

Supplemental Figure 6



Sparse double labeling of CD68(+)/TUNEL(+) cells within the infarct. Double immunostaining of CD68 (red, macrophages) and TUNEL (green, a marker of apoptosis) is assayed for *Flt4^{fl/fl}* and *Flt4^{fl/fl}; Cdh5-Cre^{ERT2}* animals at day 3 (**A-B**), day 7 (**C-D**), and day 14 (**E-F**) post-MI. Inset images are high power images of the boxes outlined in **A-F**. Yellow arrowheads demonstrate specific cells positive for both CD68 and TUNEL. Images are representative of >4 animals assayed at each time point and genotype.

Supplemental Figure 7



Ejection fraction (EF) of each genotype with animals grouped by sex. The EF data from Figures 1-3 is reproduced here, with animals separated by sex. EF 14 days after MI is graphed for mice subjected to MI after pan-endothelial deletion of VEGFR3 (**A**), lymphatic-endothelial deletion of VEGFR3 (**B**) or global deletion of both VEGF-C and VEGF-D (**C**). For each group the EF of control littermates is also shown. None of the means are statistically different in male vs. female groups. Bar graphs are centered on mean, error bars represent SEM.

SUPPLEMENTAL TABLE 1

	<i>Flt4^{fl/fl}</i>	<i>Flt4^{fl/fl}; Cdh5-Cre^{ERT2}</i>	<i>Flt4^{fl/fl}</i>	<i>Flt4^{fl/fl}; Prox1-Cre^{ERT2}</i>	<i>Vegfd^{-/-}; Vegfc^{fl/fl}</i>	<i>Vegfd^{-/-}; Vegfc^{fl/fl}; R26-Cre^{ERT2}</i>
N	18	15	6	14	6	9
EF Mean (group specific)	31.3	31.4	31.0	32.8	26.5	30.0
EF SD (group specific)	11.2	14.5	11.0	13.8	6.2	14.0
Variance (group specific)	125.44	210.25	121	190.44	38.44	196
EF Differences* (study specific)	0.1		1.8		3.5	
Mean of 3 EF Differences	1.8					
Var of Mean EF Differences (V_i)**	20.99		33.77		28.18	
Var of 3 mean EF Differences (V)***	2.89					
Weight = 1/(V_i + V)	0.041		0.027		0.032	
	Weighted Mean		1.64			
	Variance of combined effect		9.87			
	Std. Error of combined effect		3.14			

Meta analysis parameters and calculated values used for random-effects model as described in Borenstein (30).

* The experiment-specific EF difference is the difference between the experimental and control groups for each experiment

**The variance of an experiment-specific EF difference is the sum variances of the two group means.

***The variance of the experiment-specific mean EF differences

