Supplemental Materials

Title: Transfusion of Adult, but not Neonatal, Platelets Promotes Monocyte Trafficking in Neonatal Mice

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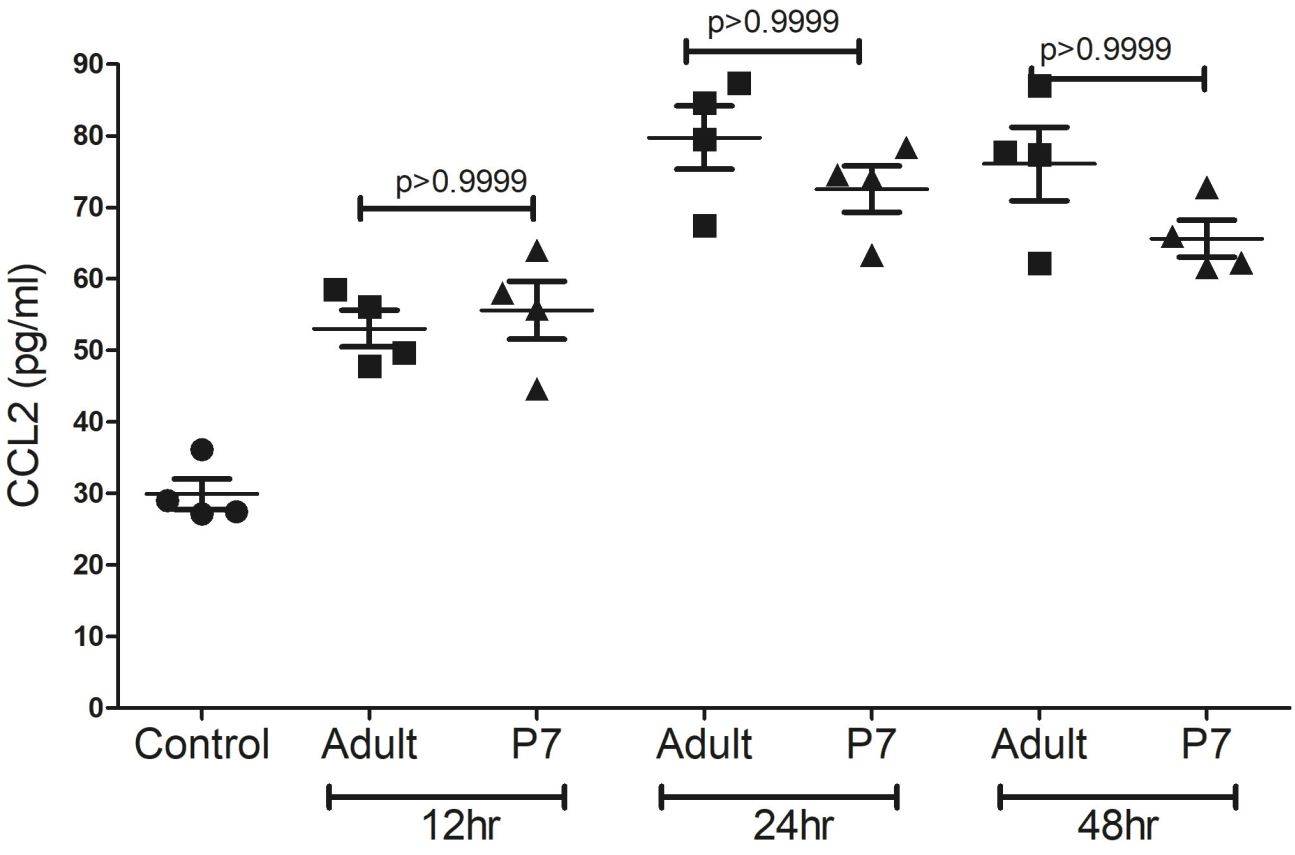

Figure S1: Adult or P7 platelets were co-incubated with monocytes and 12, 24 and 48 hr later culture supernatant was collected to measure CCL2 by ELISA. Both adult and P7 platelets induced CCL2 production *in vitro* over 48 hrs *in vitro* (mean ± SEM , Kruskal Wallis with Dunn's multiple comparison).

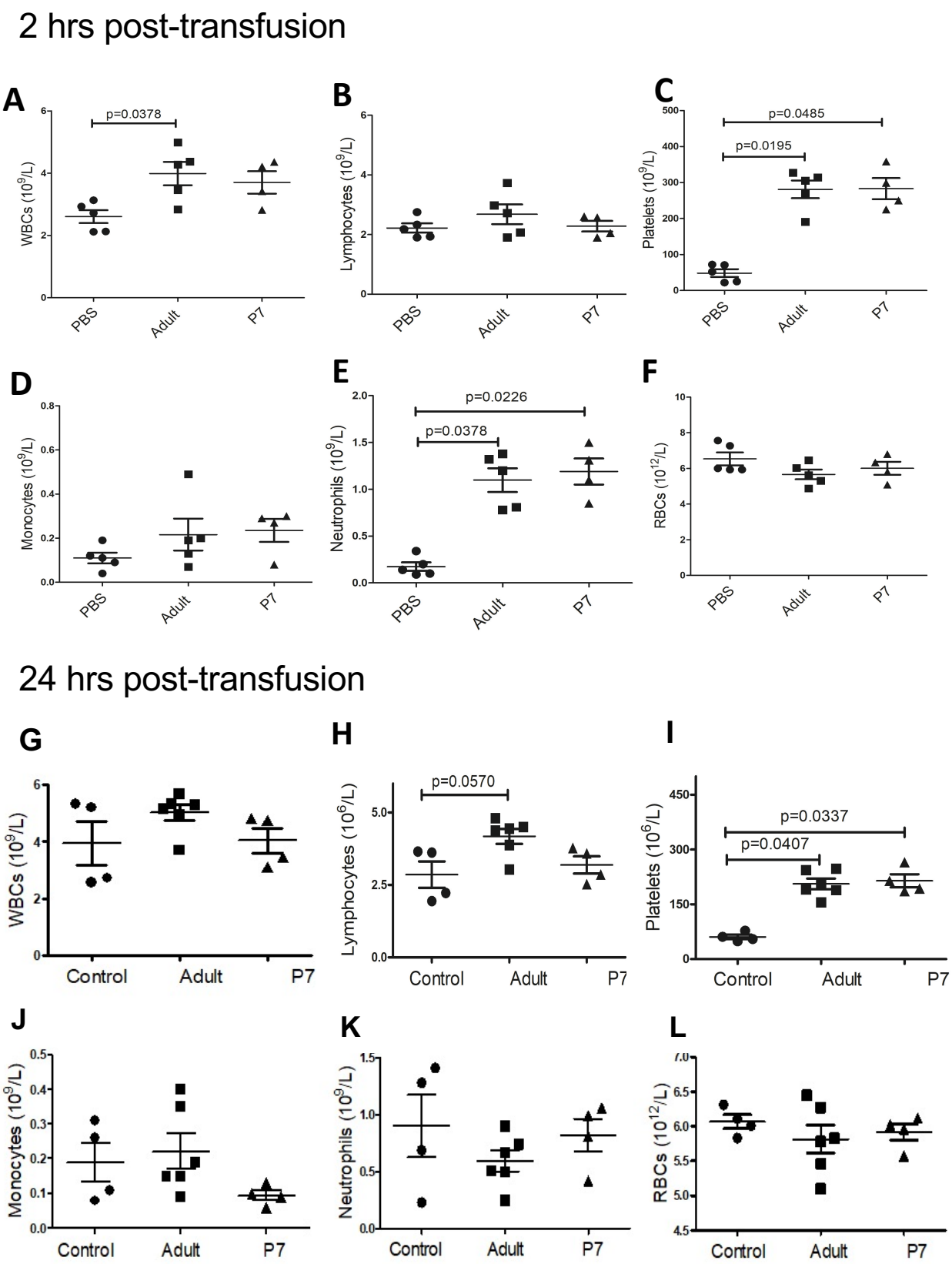

Figure S2: TPOR$^{-/-}$ mice were transfused with adult or P7 platelets and 2 or 24 hrs later CBC were performed. Adult and P7 platelets had similar post-transfusion CBC at both time points (mean ± SEM, Kruskal Wallis with Dunn's multiple comparison).

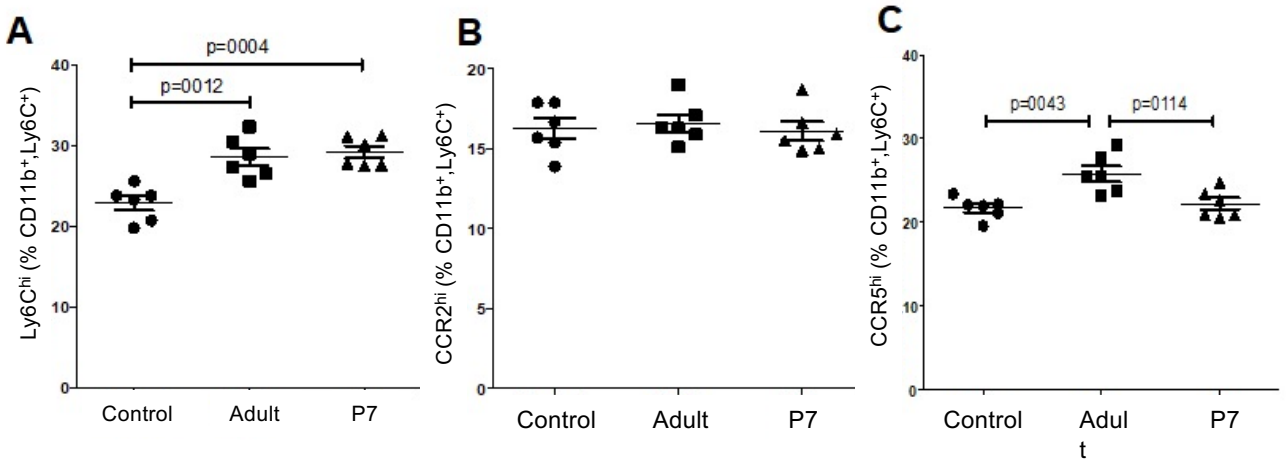

Figure S3: Platelet releasates did not increase trafficking monocyte phenotype *in vitro*. BMM were co-incubated with adult or P7 platelet releasates and 4 hrs later Ly6C, CCR2 and CCR5 expression were determined. A) Both adult and P7 releasates increased Ly6C but B) neither increased CCR2 and C) only adult increased CCR5 (mean ± SEM 1-way ANOVA with Bonferroni correction).

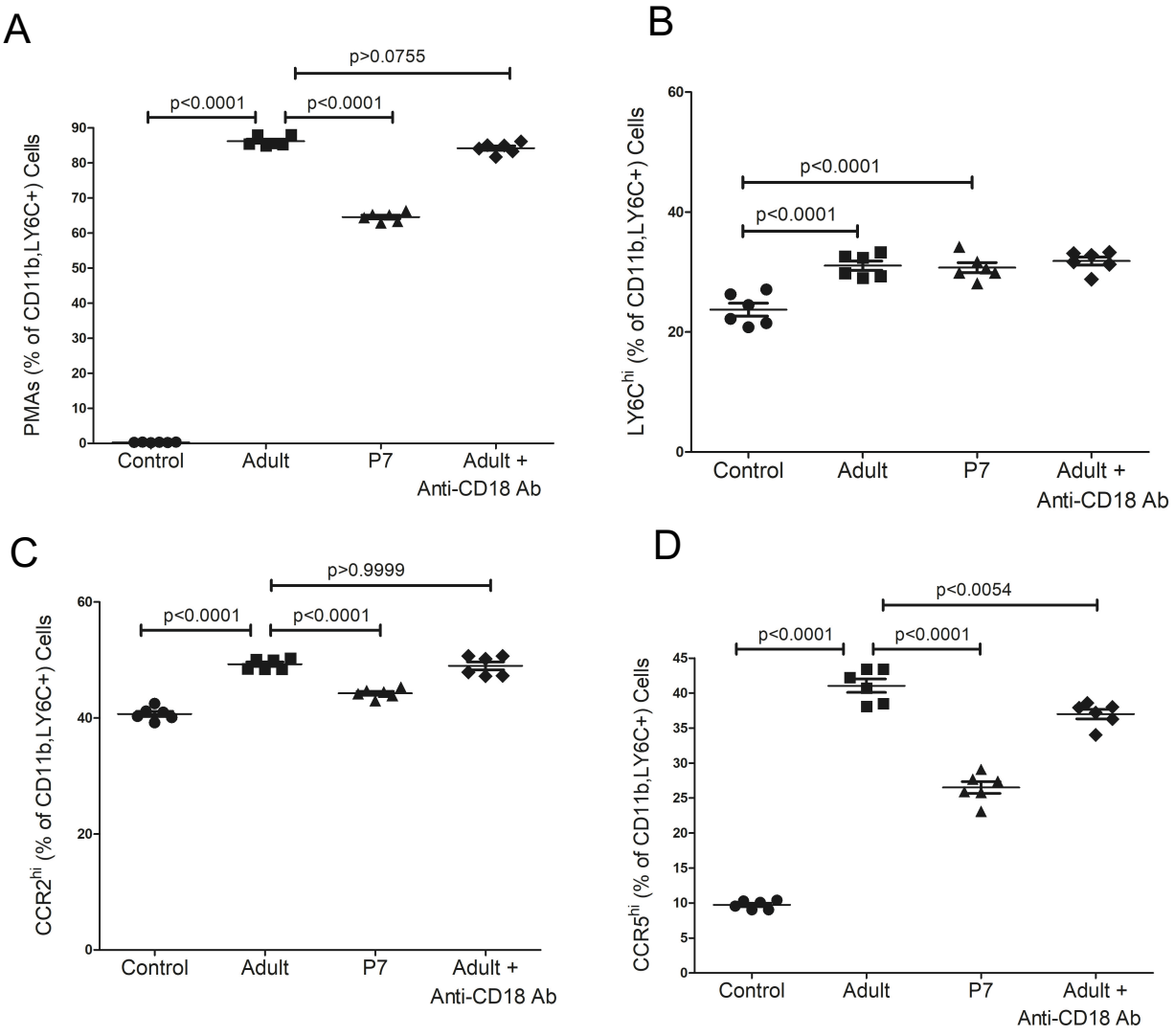

Figure S4: GP1bα-CD18 interactions are not responsible for monocyte trafficking phenotypes. Adult or P7 platelets were co-incubated with monocytes in the presence of IgG or anti-CD18 Ab. 4 hrs later PMAs, Ly6C, CCR2 and CCR5 expression were determined. Anti-CD18 had little effect on A) PMAs, B) Ly6C, or C) CCR2 expression. However, anti-CD18 Ab limited adult platelet induced CCR5 expression. (mean ± SEM 1-way ANOVA with Bonferroni correction).

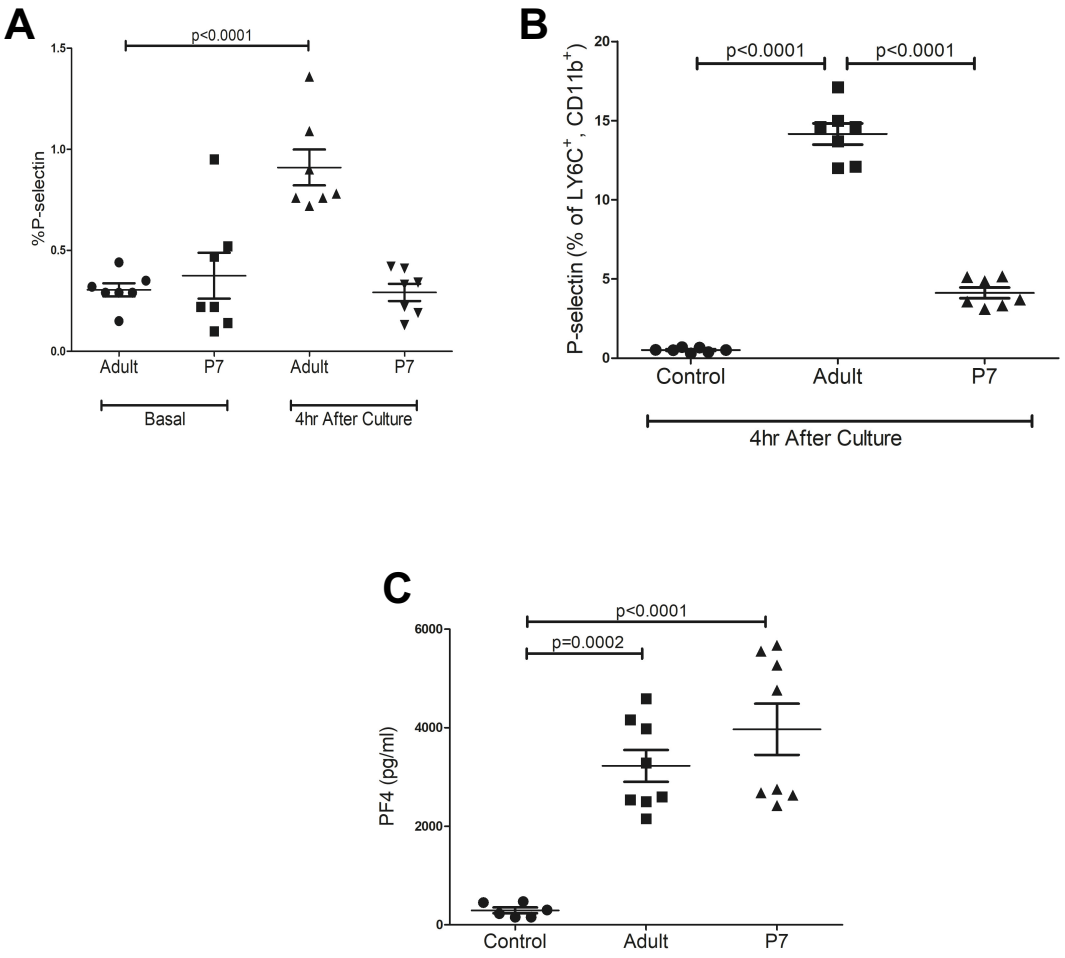

Figure S5: P-selectin expression before and after platelet-monocyte incubation *in vitro*. Primary mouse BMM were co-incubated with adult or P7 platelets and 4 hrs later P-selectin expression on platelets and monocytes was determined. A) Adult platelets had more P-selectin expression after 4 hrs in culture. B) P-selectin expression on monocytes co-incubated with adult platelets after 4hr was increased. C) Supernatant PF4 was the same for both adult and P7 platelets (mean ± SEM 1-way ANOVA with Bonferroni correction).

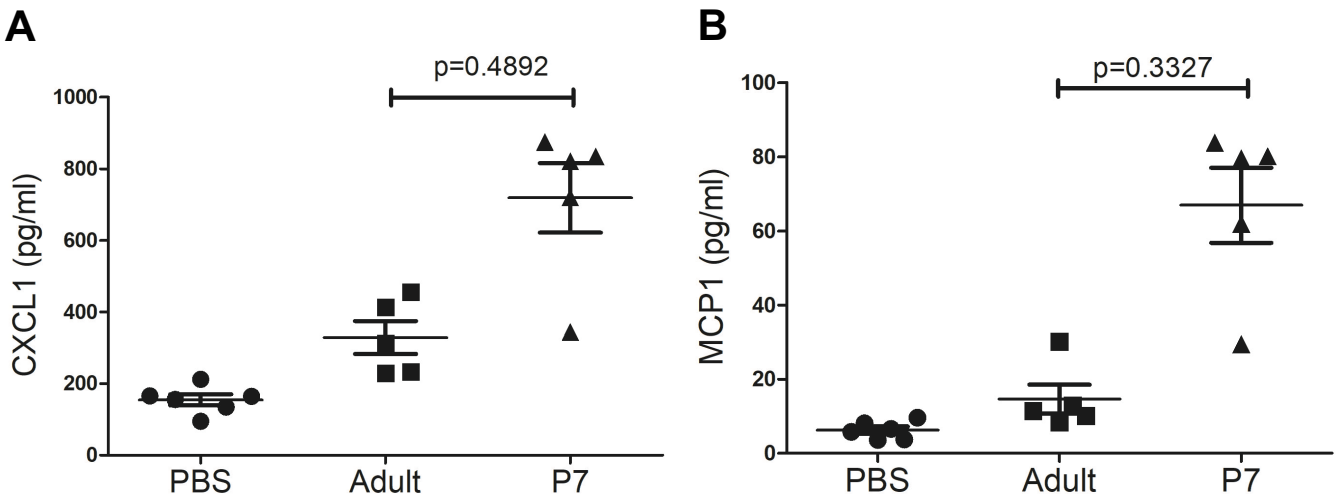

Figure S6: TPOR$^{-/-}$ mice were transfused with adult or P7 platelets and 4hr later plasma was collected. ELISAs for CXCL1 and CCL2 were performed. A) Both adult and P7 transfusions increased plasma CXCL1 and B) P7 platelets increased CCL2 more than adult platelets (mean ± SEM , Kruskal Wallis with Dunn's multiple comparison).

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse	Jax Labs	C57Bl6/J	Male	https://www.jax.org/strain/000664?featured
Mouse	Jax Labs	C57Bl6/J	Female	https://www.jax.org/strain/000664?featured
Mouse	Envigo	ICR-Cd1	Male and female	https://www.envigo.com/model/hsd-icr-cd-1

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	Mouse	Jax	C57Bl6/J	*Mpl KO*	https://www.jax.org/strain/005124
Parent - Female	Mouse	Jax	C57Bl6/J	*Mpl KO*	https://www.jax.org/strain/005124

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration
LY6C	BioLegend	128026	1µg/ml
CD11B	BioLegend	101212	1µg/ml
CD41	BioLegend	133915	1µg/ml
CCR2	BioLegend	150610	1µg/ml
CCR5	BioLegend	107016	1µg/ml
CD45	BioLegend	103108	1µg/ml
LY6G	BioLegend	127612	1µg/ml
P-selectin	Fisher Scientific	BDB553744	1µg/ml
Prostaglandin I2	Cayman Chemical	18220	5nM/ml
Anti-mouse PSGL-1	BioXcell	BE0186	20mg/kg
Anti-mouse CD18	BioXcell	BE0009	40µg/ml
IgG	BioXcell	BP0091	10mg/kg
Mouse CCL2/JE/MCP-1 DuoSet ELISA	R&D system	DY479-05	As per instruction
Mouse CXCL1/KC DuoSet ELISA	R&D system	DY453-05	As per instruction
B2M ELISA Kit (Mouse)	Aviva Biosystem	OKEH00344	As per instruction
Human beta 2-Microglobulin Parameter Assay Kit	R&D system	KGE019	As per instruction
Recombinant Mouse CCL2/JE/MCP-1 Protein	R&D system	479-JE-050/CF	200 nM/ml
Recombinant Mouse CCL5/RANTES Protein	R&D system	478-MR-025/CF	200 nM/ml
Mouse Monocytes isolation kit	STEMCELL Technologies	19861	As per instruction
Crystal Violet	Fisher Scientific	C581-25	1%

Primer

Target gene	Vendor or Source	Catalog #	Species
Ccl2	Thermo Fisher	Mm00441242_m1	Mouse
Ccr2	Thermo Fisher	Mm99999051_gH	Mouse

DOI [to be added]

Ccr5	Thermo Fisher	Mm01963251_s1	Mouse
Gapdh	Thermo Fisher	Mm99999915_g1	Mouse

ARRIVE GUIDELINES

Study Design

Groups	Sex	Age	Number (prior to experiment)	Number (after termination)	Littermates (Yes/No)	Other description
Group 1	M/F	8-10 wks	Not available	Not available	Yes	Adult platelet and monocyte donor
Group 2	M/F	P7	Not available	Not available	Yes	Neonatal platelet donor
TPOR$^{-/-}$	M/F	P14	Not available	Not available	Yes	Transfusion recipient

Sample Size: Not available

Inclusion Criteria: Not applicable

Exclusion Criteria: Not applicable

Randomization: Not applicable – platelet donors and transfusion recipients must be of the appropriate age and must either be WT donors or TPOR$^{-/-}$ transfusion recipients. Whether transfusion recipients received adult or neonatal transfusions was random.

Blinding: Not applicable – platelet donors and transfusion recipients must be of the appropriate age and must either be WT donors or TPOR$^{-/-}$ transfusion recipients.

DOI [to be added]