

Supplementary Materials for

Optimization of synoviocyte-targeted biologic for inflammatory arthritis in combination or bispecific administration with TNF inhibitors

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This PDF includes:

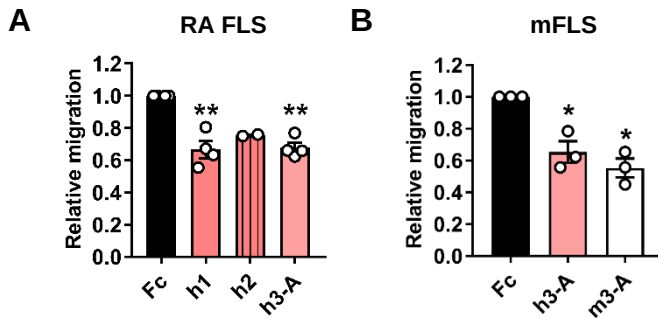
Supplemental Figure 1. Effect of varying linkers and species in inhibiting migration.

Supplemental Figure 2. Effect of Ig1&2-Fc in inhibiting scratch wound migration.

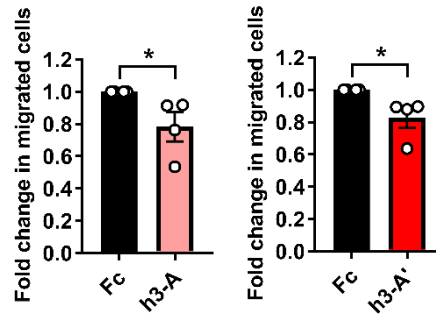
Supplemental Figure 3. Detection of Ig1&2-Fc by anti-PTPRS Fab.

Supplemental Figure 4. Therapeutic treatment of bispecific Ig1&2 fusion to mTnfr2.

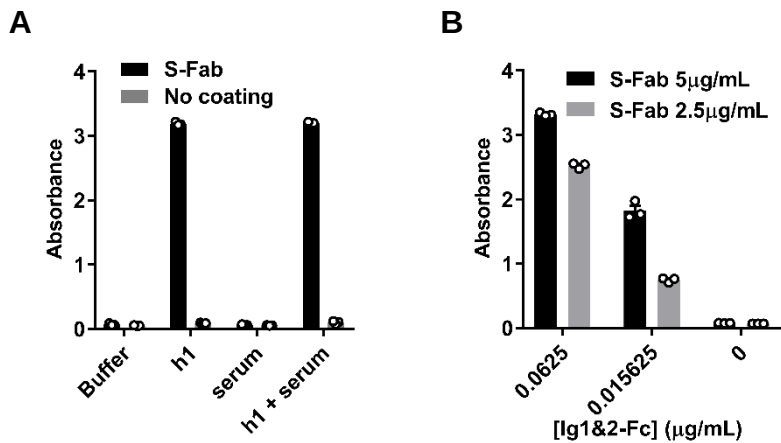
Supplemental Table 1. Gross necropsy of mice from four-month toxicology study.



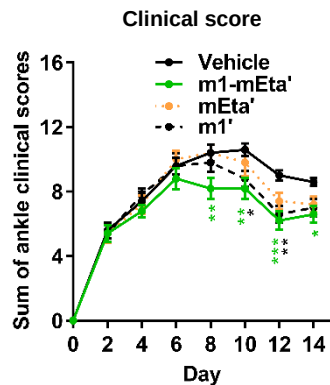
Supplemental Figure 1. Effect of varying linkers and species in inhibiting migration. (A) Relative transwell migration of RA FLS in response to 10% FBS in the presence of Ig1&2-Fc or Fc control protein (50 nM) (**h2**: $n = 2$; others: $n = 4$). **(B)** Relative transwell migration of mFLS in response to 10% FBS in the presence of Ig1&2-Fc or Fc control protein (100 nM) ($n = 3$). **(A and B)** Mean $\pm$ SEM relative to Fc control sample is shown. * $P < 0.05$ and ** $P < 0.01$ by unpaired t test with Welch's correction **(A and B)**.



Supplemental Figure 2. Effect of Ig1&2-Fc in inhibiting scratch wound migration. Relative migration of RA FLS cells past scratch wound boundary in response to 5% FBS in the presence of Ig1&2-Fc or Fc control protein (100 nM) ($n = 4$). Mean $\pm$ SEM relative to Fc control sample is shown. * $P < 0.05$ by Mann-Whitney U test.



Supplemental Figure 3. Detection of Ig1&2-Fc by anti-PTPRS Fab. (A) Ig1&2-Fc protein in the presence or absence of mouse serum matrix was detected by coating with **S-Fab**. (B) Increased sensitivity of detection of Ig1&2-Fc was achieved by coating with a lower concentration of **S-Fab**. (A and B) Mean $\pm$ SEM of triplicate wells is shown.



Supplemental Figure 4. Therapeutic treatment of bispecific Ig1&2 fusion to mTnfr2. Therapeutic treatment of BALB/c mice with K/BxN STIA by intraperitoneal injection of 0.3 mg (12 mg/kg) **m1-mEta'**, 0.1 mg (4 mg/kg) **mEta'**, 0.2 mg (8 mg/kg) **m1'**, or vehicle every 2 days for a total of 7 injections ($n = 5$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ by two-way ANOVA with Dunnett's correction.

Supplementary Table 1. Gross necropsy of mice from four-month toxicology study		
<u>Description</u>	<u>(PBS)</u>	<u>Ig1&2-Fc (0.1 mg)</u>
Skin		
No obvious abnormalities	8/9	10/10
Rare focus of mast cells	1/9	- -
Heart		
No obvious abnormalities	4/9	4/10
Abnormal eosinophilic cardiomyocytes	4/9	4/10
Capillary congestion	1/9	2/10
Lungs		
Congestion*	2/9	1/10
Collapse*	4/9	4/10
Hemorrhage	5/9	5/10
Few foci of inflammation	2/9	3/10
Foci of inflammation	1/9	2/10
Few foci of pulmonary edema	- -	1/10
Adenoma	1/9	1/10
*Observed congestion and collapse may be due to lung fixation during processing		
Liver		
Rare foci of portal and parenchymal inflammation	3/9	3/10
Rare foci of portal or parenchymal inflammation with capillary congestion	- -	2/10
Mild portal inflammation with or without minimal foci of parenchymal inflammation	3/9	3/10
Bridging portal inflammation	1/9	- -
Very little inflammation	2/9	2/10
Kidney		
No obvious abnormalities	6/9	7/10
Focus of inflammation	2/9	1/10
Not seen in section	1/9	2/10
Bladder		
No obvious abnormalities	7/9	7/10
Not seen in section	2/9	3/10

Spleen		
Extra medullary hematopoiesis	9/9	10/10
Thymus		
No obvious abnormalities	9/9	10/10
Salivary glands		
No obvious abnormalities	9/9	10/10
Stomach		
No obvious abnormalities	8/9	10/10
Focus of inflammation	1/9	- -
Pancreas		
No obvious abnormalities	8/9	7/10
Abnormal foci in exocrine glands	- -	1/10
Rare foci of pale exocrine glands	- -	1/10
Rare abnormal foci in parenchyma	- -	1/10
Abnormal vacuoles in exocrine glands	1/9	- -
Small intestine (duodenum & ileum)		
No obvious abnormalities	6/9	7/10
Polyp	1/9	1/10
Focus of inflammation	1/9	- -
Polyp; inflammation	- -	1/10
Enlarged Peyer's patch	1/9	- -
Peyer's patch reactive with prominent macrophages	- -	1/10
Prostate		
No obvious abnormalities	3/4	3/5
Not seen in section	1/4	2/5
Seminal vesicles		
No obvious abnormalities	4/4	4/5
Inflammation	- -	1/5
Polyp, hyperplasia	- -	- -
Ovary		
Epithelial inflammation	3/5	3/5
Not seen in section	2/5	2/5
Uterus		

No obvious abnormalities	2/5	2/5
Dilated	- -	- -
Not seen in section	3/5	3/5
Cervix		
Epithelial inflammation	3/5	4/5
Not seen in section	2/5	1/5