Supplemental Materials and Methods

SUPPLEMENTAL METHODS

WESTERN BLOT

Mouse skeletal muscle. Soleus, EDL, and gastrocnemius muscles were surgically isolated from mice and flash frozen in liquid nitrogen. 200μL of RIPA buffer was added and the muscle mechanically disrupted. Protein concentration was quantified by Bradford assay and 35μg of skeletal muscle homogenate was loaded into each well of a 12% SDS-PAGE gel and separated by electrophoresis and transferred to nitrocellulose for 1 hour at 100V. Membranes were blocked for 1 hour using 5% non-fat dry milk, incubated overnight with a custom polyclonal anti-TRIM72 antibody described in methods, and 1 hour with anti-rabbit HRP secondary antibody (Cell Signaling, catalog # 70745). Bands of interest were visualized by chemiluminescence and fold change in TRIM72 protein levels evaluated by densitometry using ImageJ.

Patient biopsies. IIM subject skeletal muscle biopsies were recovered by removing embedded biopsies from OCT on dry ice under a dissecting microscope with a scalpel to minimize OCT contamination in downstream processing. Liberated biopsies were mechanically dissociated in RIPA buffer

and changes in TRIM family proteins were evaluated by western blot as described above.

Mouse serum CK levels. 3μL of serum from sham adoptive transfer mice and mice receiving adoptive transfer of lymphocytes from *Foxp3*$^{-/Y}$*/Syt7*$^{-/-}$ mice were separated by SDS-PAGE and probed for CK levels using a polyclonal CK antibody (Invitrogen, catalog # PA5-95546) and anti-rabbit HRP secondary antibody (Cell Signaling, catalog # 70745). Bands of interest were visualized by chemiluminescence and protein levels evaluated by densitometry using ImageJ.

Mouse serum TRIM72 protein levels. 3μL of serum from sham adoptive transfer mice and mice receiving adoptive transfer of lymphocytes from *Foxp3*$^{-/Y}$*/Syt7*$^{-/-}$ mice were separated by SDS-PAGE and probed for circulating levels of TRIM72 protein using the polyclonal TRIM72 antibody described in methods and anti-rabbit HRP secondary antibody (abcam, catalog # ab205719). Bands of interest were visualized by chemiluminescence.

WESTERN BLOT AUTOANTIBODY SCREENS

Mouse serum. 35μg of liver homogenate (negative control), tibialis anterior (positive control), and recombinant human TRIM72 protein (10 and

20ng) were separated by electrophoresis and transferred to nitrocellulose. Membranes were incubated with a 1:200 dilution of serum collected from adoptive transfer mice (*Foxp3*$^{-/Y}$*/Syt7*$^{-/-}$ double mutant or *Foxp3*$^{-/-}$), C57Bl mice, or purified polyclonal antibody against TRIM72. Membranes were then incubated with secondary antibodies conjugated with horseradish peroxidase and images acquired by chemiluminescence.

Subject serum. HEK293 cells were transfected with eGFP or TRIM family clones of interest. Lysates were collected and incubated overnight with protein A/G beads to immuno-pull-down TRIM proteins of interest. Enriched lysates were then separated by electrophoresis and transferred to nitrocellulose membranes and probed for autoantibodies using IIM subject serum as the primary antibody.

ROTATION DAMAGE ASSAY

1 x 10^5 HEK293 cells were seeded in 2mL flat bottom microcentrifuge tubes in 500μL of DMEM complete media and allowed to adhere overnight. Media was aspirated from each tube, cells washed 3x with PBS, and 200μL of Tyrode's buffer supplemented with 2mM Ca^{2+} was added to each tube. 20μL (7.23mg ± 0.63mg) of ≤ 106μm glass beads are added by pipetting directly from

the dry stock. Tubes were then placed in a hematological mixer and rotated end over end at 15 RPM for 1 minute. Following rotation, tubes were removed from the mixer and 10μL of supernatant transferred to a 96 well plate. LDH release was measured to determine membrane resealing capacity.

ROLLING GLASS BEAD ASSAY

C2C12 myoblasts were stimulated to differentiate into myotubes by serum starvation. After 5-7 days of serum starvation, myotubes were washed 3X with PBS and 500μL Tyrode's solution supplemented with 2mM Ca^{2+} added to each well. 600μm silica beads were gently added down the side of each well using the cap from a 200μL PCR tube. This method reliably delivered 98mg+/-10mg of silica beads to each well. A 2% Triton solution was used as a positive control (100% LDH release), wells agitated without the addition of silica beads (background LDH/negative control), myotubes injured in the presence of normal rabbit serum, or myotubes injured with a 1:100 or 1:200 dilution of anti-TRIM72 antibodies. Plates were agitated on an orbital shaker for 1 minute at 160 rpm to damage membranes and allowed to reseal for 2 minutes. After resealing, the supernatant was collected from the plates and LDH release was measured to determine membrane resealing capacity.

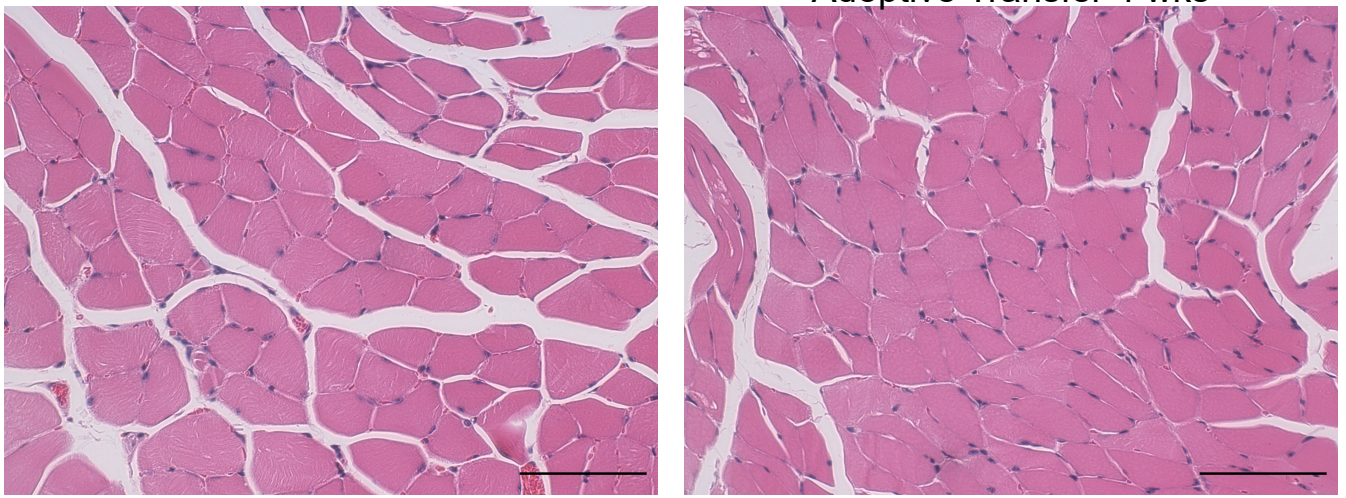
BALLISTIC INJURY ASSAY

C2C12 myoblasts were differentiated into myotubes as described above in 35mm tissue culture plates. Myotubes were washed 3X with PBS and 1mL Tyrode's solution supplemented with 2mM Ca^{2+} was added to each plate. 2% Triton solution (total LDH release), Healthy subject serum, or anti-TRIM72+ IIM subject serum was diluted 1:200 in Tyrodes's solution supplemented with 2mM Ca^{2+} and added to each plate. Plates were allowed 2 minutes to equilibrate at 37^{o}C. Myotubes exposed to Tyrode's only were used to control for damage caused by helium pressure wave. Immediately before ballistic injury, Tyrode's solution was removed and reserved from each 35mm plate and the myotubes were injured with 1.0μm gold particles in a BioRad PDS-1000/He biolistic particle delivery system. Immediately following injury, reserved Tyrode's solution was gently added back to each plate and myotubes allowed to reseal for 2 minutes. The supernatant was collected from injured plates and LDH release measured to determine membrane resealing capacity.

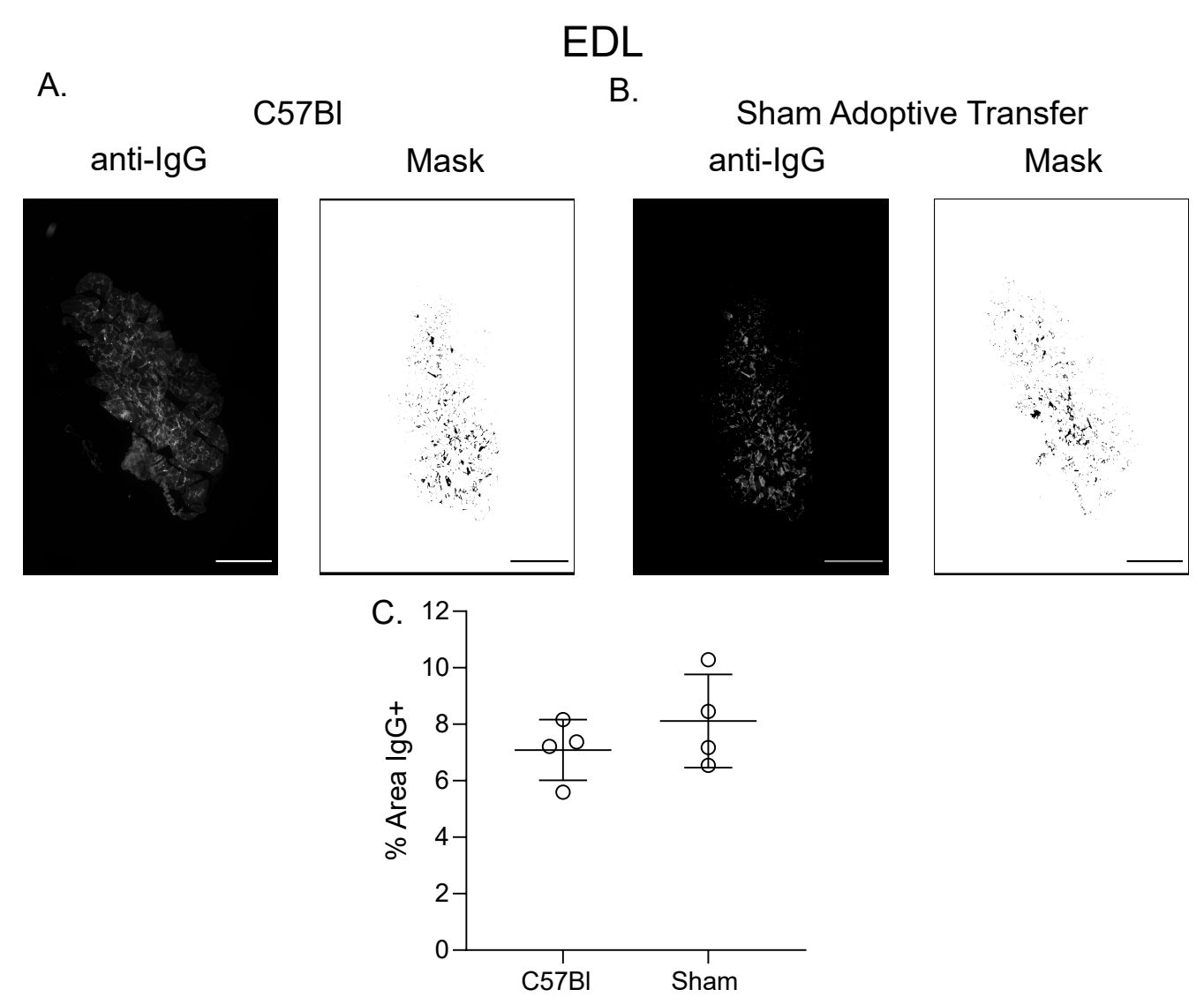
Soleus

A. Sham 4wks

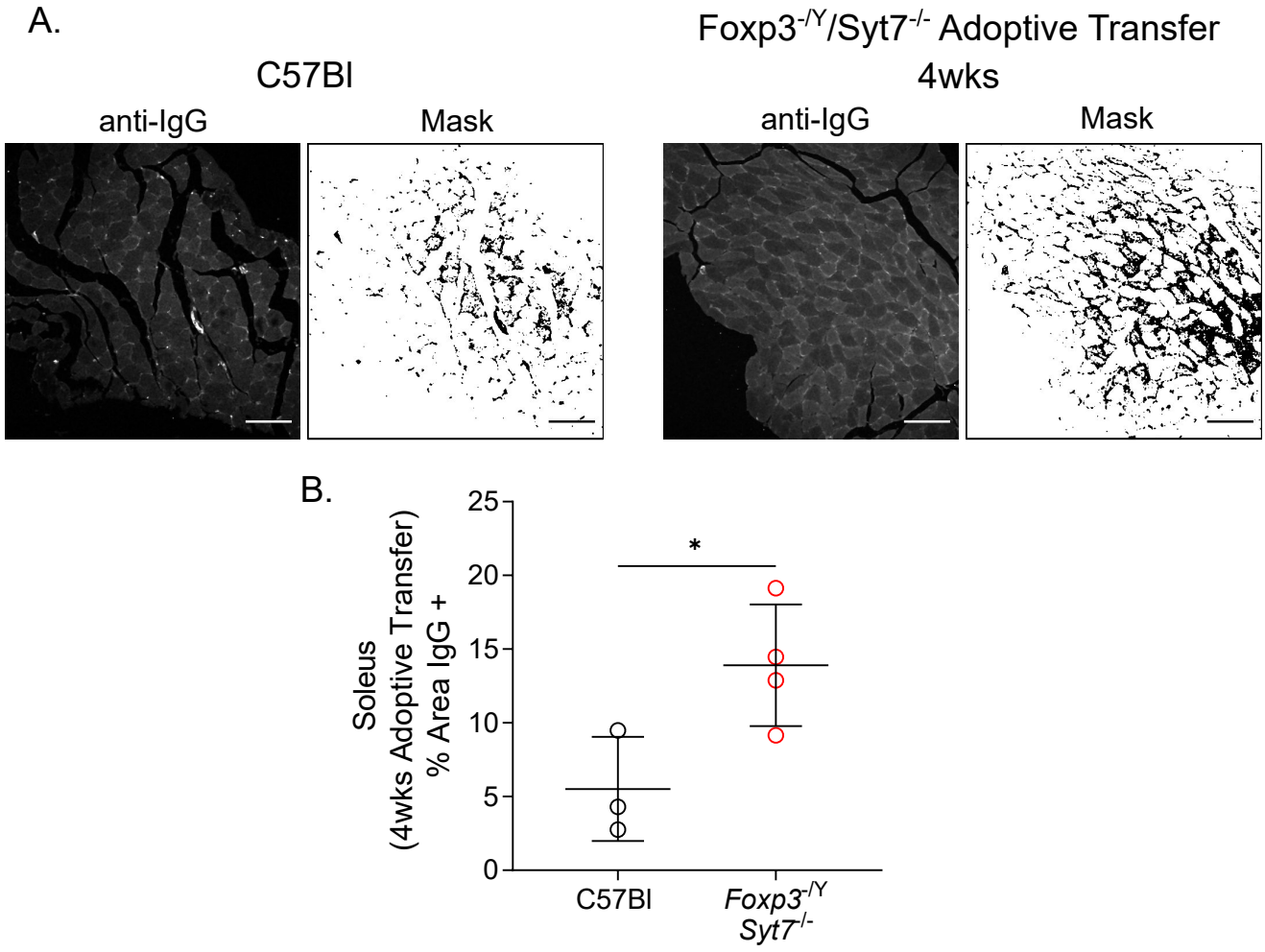
B. Foxp3$^{-/Y}$/Syt7$^{-/-}$ Adoptive Transfer 4 wks



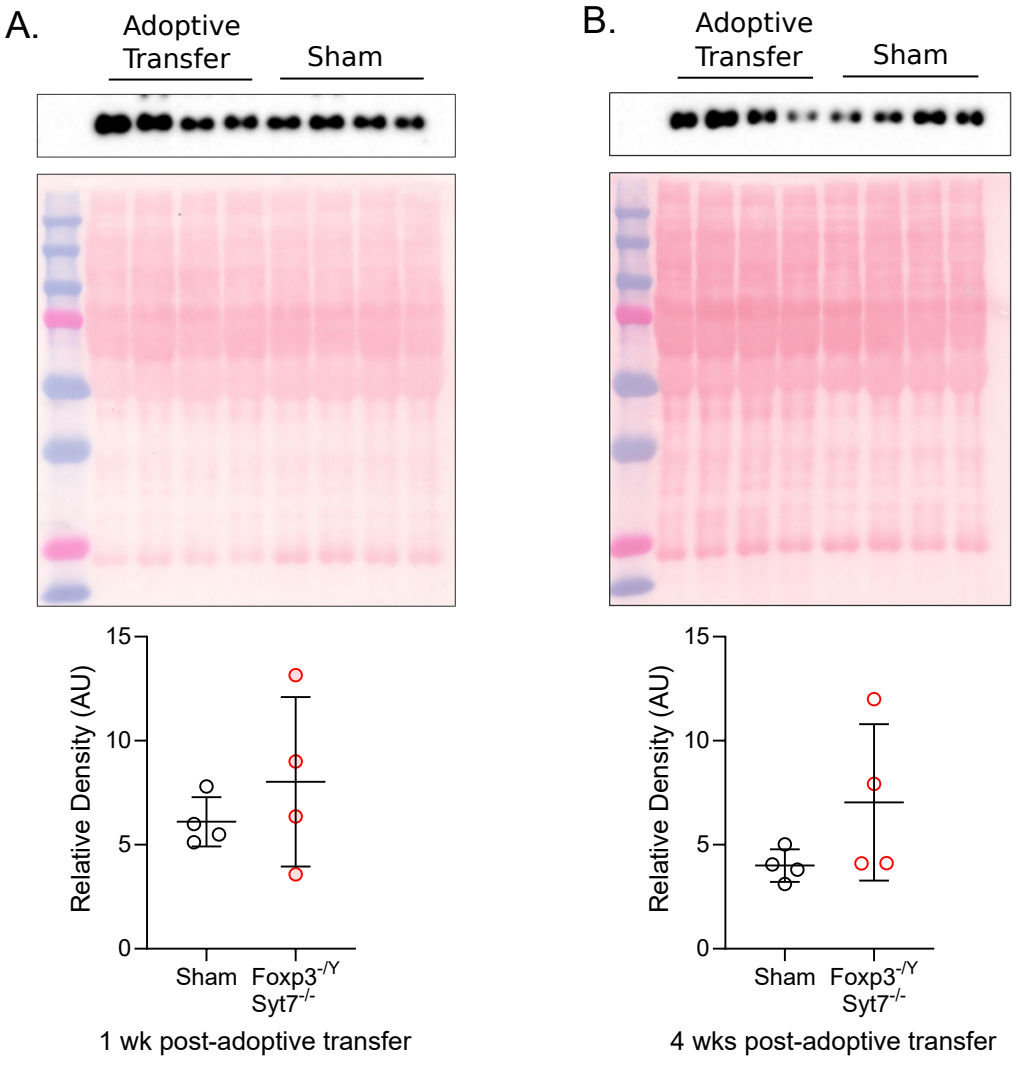
Supplemental Fig. 1. Distal skeletal muscle (soleus) is spared from inflammation in an adoptive transfer model of IIM. Representative images of H&E stained soleus muscles from Rag1$^{-/-}$ mice receiving sham adoptive transfer or adoptive transfer of lymphocytes from Foxp3$^{-/Y}$/Syt7$^{-/-}$ mice. Rag1$^{-/-}$ mice receiving adoptive transfer of lymphocytes from the Foxp3$^{-/Y}$/Syt7$^{-/-}$ background have no significant areas of inflammation. A.) Soleus muscle from sham adoptive transfer mice at 4 weeks following adoptive transfer. B.) Soleus muscle from adoptive transfer mice at 4 weeks following adoptive transfer. Scale bars represent 200μm.



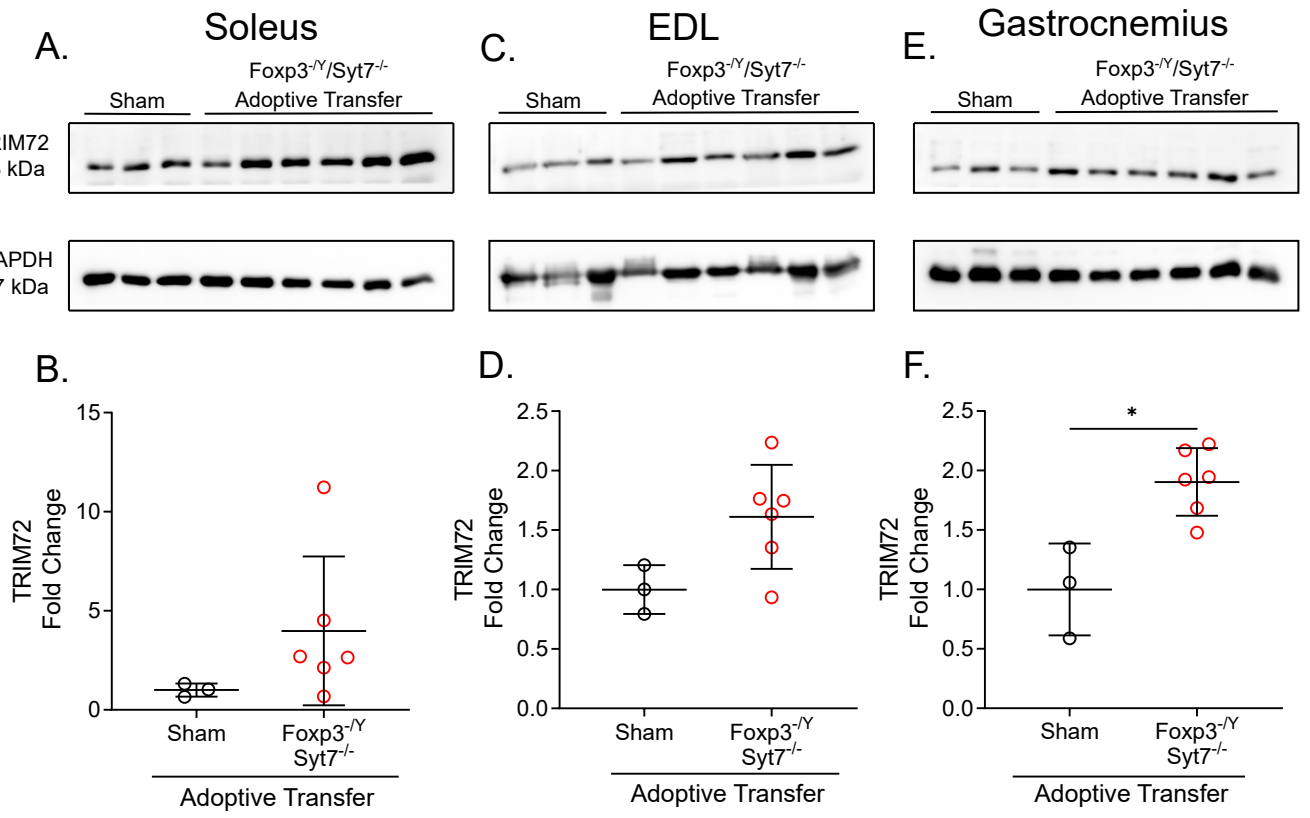
Supplemental Fig. 2. Quantification of background IgG signal in C57Bl versus sham adoptive transfer to Rag1$^{-/-}$ mice. C57Bl mice were chosen as IgG staining controls due to the lack of B-cells in Rag1$^{-/-}$ mice. IgG staining of Rag1$^{-/-}$ mice receiving sham adoptive transfer was performed on distal EDL muscles to determine non-specific background staining and found to be comparable to C57Bl skeletal muscle. A.) Representative images of C57Bl EDL muscle and B.) Rag1$^{-/-}$ mice receiving sham adoptive transfer. Fluorescent image (Anti-IgG), Threshold image used in quantification (Mask). C.) Quantification of background levels of nonspecific IgG staining in C57Bl and Rag1$^{-/-}$ sham adoptive transfer mice (n=4; (t,df)=1.043, 6); p=0.3371). Scale bars represent 200μm.



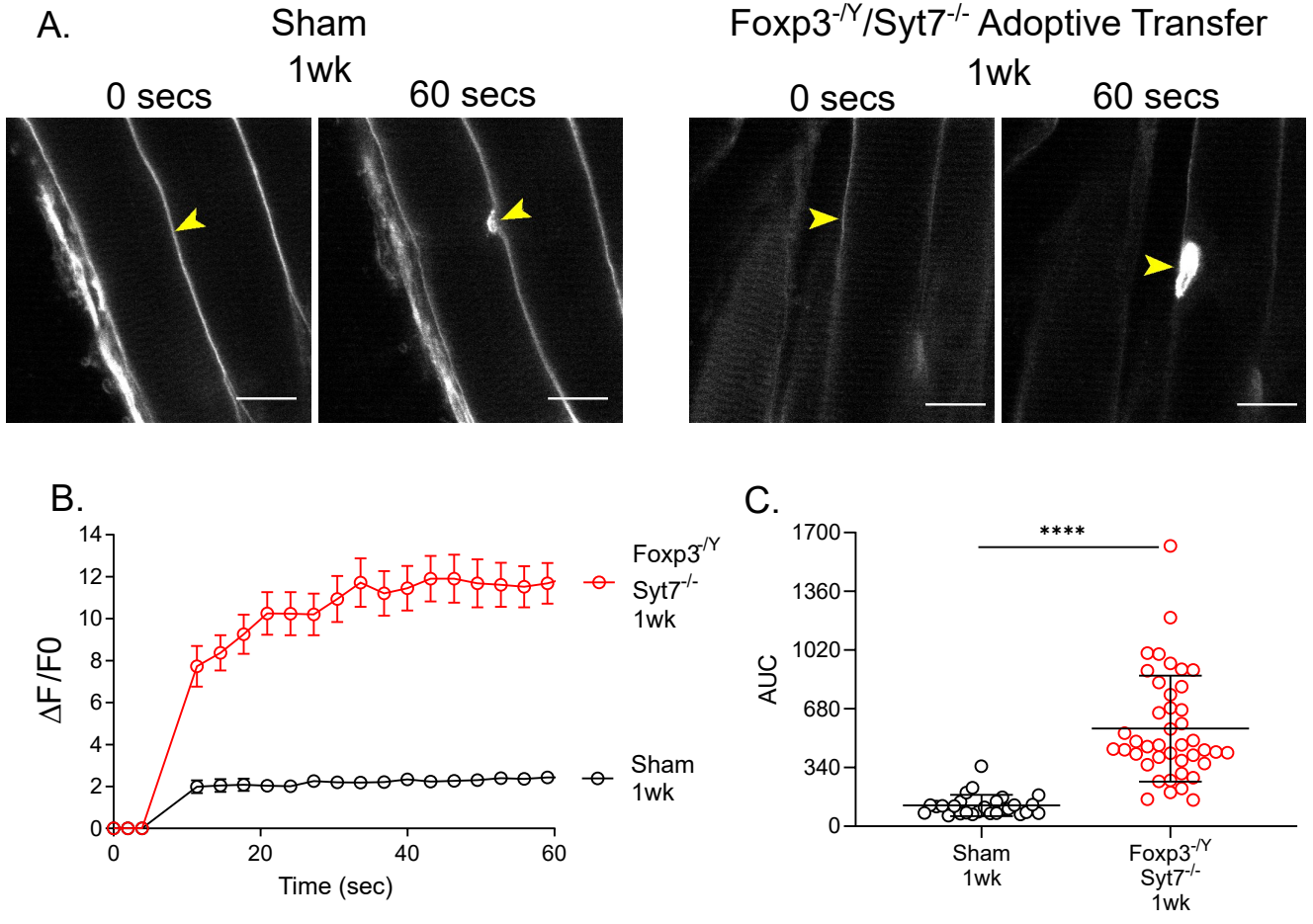
Supplemental Fig. 3. Membrane integrity is significantly reduced in soleus skeletal muscles in a murine adoptive transfer model of IIM. A.) Representative images of IgG immunohistochemical labeling of soleus muscle from C57Bl mice and Rag1$^{-/-}$ receiving adoptive transfer of lymphocytes from the Foxp3$^{-/Y}$/Syt7$^{-/-}$ background. B.) Quantification of % area soleus skeletal muscle positive for IgG. n=3, 4 for C57Bl and adoptive transfer respectively; (t,df)=2.815, 5; p=0.0373. Scale bars represent 200μm.



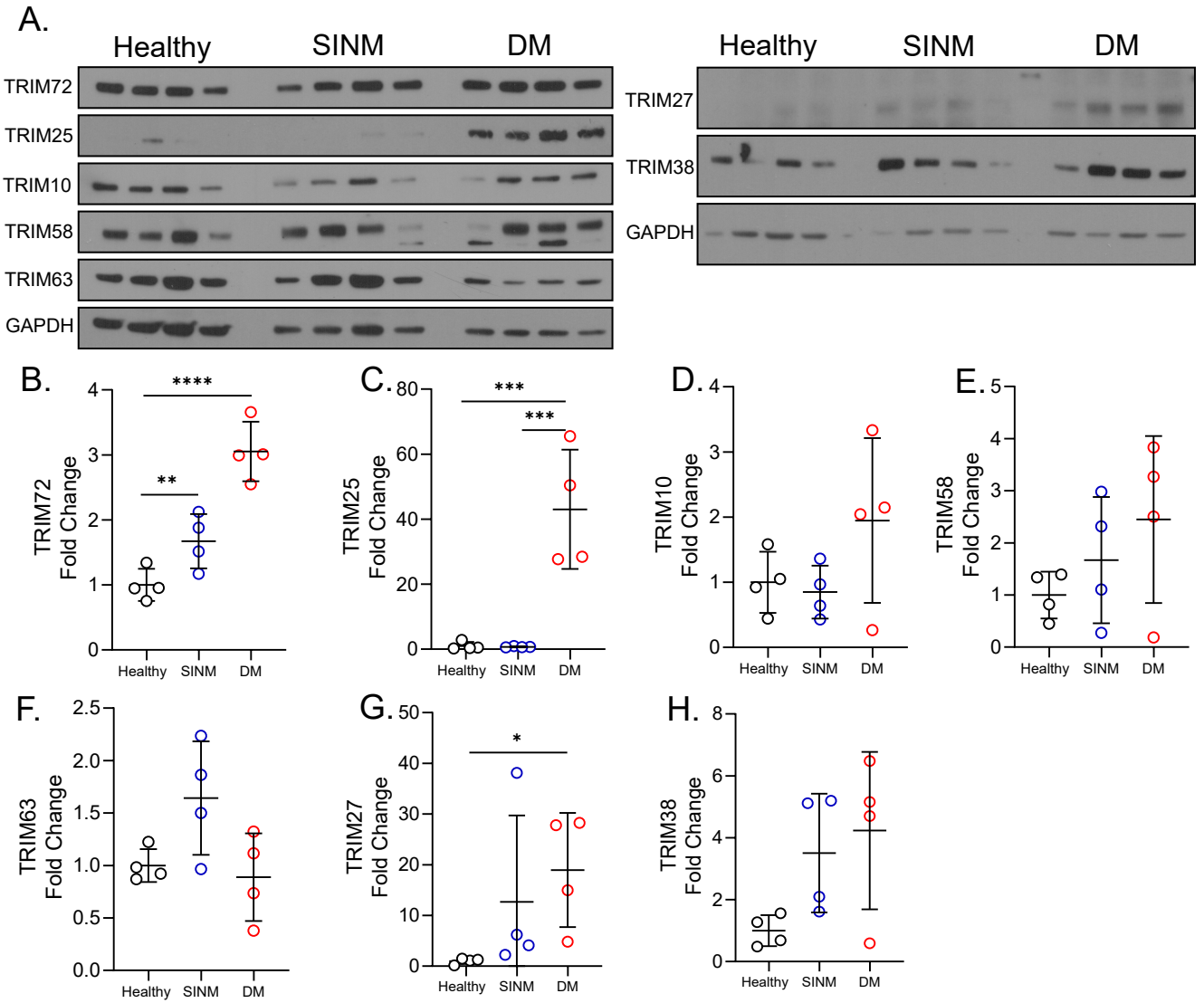
Supplemental Fig. 4 Western blot and quantification of serum CK levels in adoptive transfer mice. Equal volumes (3 mL) of serum from mice receiving adoptive transfer of lymphocytes from $Foxp3^{-/Y}/Syt7^{-/-}$ mice or mice receiving sham adoptive transfer separated by SDS-PAGE and quantified by western blot. A.) 1 week post-adoptive transfer. B.) 4 weeks post-adoptive transfer.



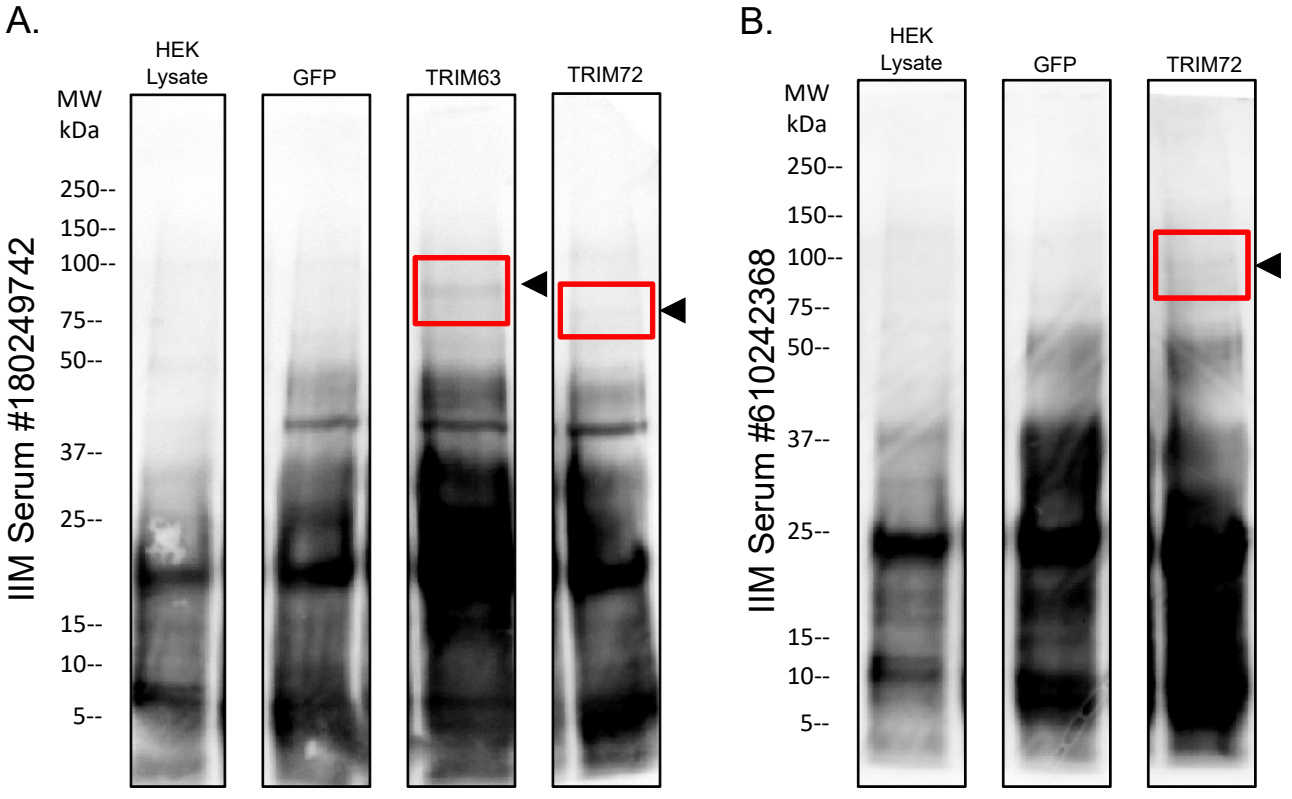
Supplemental Figure 5. TRIM72 Protein Levels in Rag1$^{-/-}$ mice adoptively transferred with lymphocytes from the Foxp3$^{-/Y}$/Syt7$^{-/-}$ double mutant background. Indicated skeletal muscle homogenates isolated from Rag1$^{-/-}$ mice receiving sham adoptive transfer or adoptive transfer of lymph node preparations from Foxp3$^{-/Y}$/Syt7$^{-/-}$ mice screened 4 weeks post adoptive transfer for changes in TRIM72 protein expression by western blot. Graphs depict fold change in TRIM72 protein levels in A&B.) Soleus muscle (mean ± SEM=1 ± 0.1904, n=3, and 3.986 ± 1.534, n=6; t,df=1.328, 7; p=0.2259 for sham and Foxp3$^{-/Y}$/Syt7$^{-/-}$ adoptive transfer 4wks respectively. C&D). Extensor digitorum longus muscle (mean ± SEM=1 ± 0.1185, n=3 and 1.612 ± 0.1789, n=6; t,df=2.241, 7; p=0.06 for sham and Foxp3$^{-/Y}$/Syt7$^{-/-}$ adoptive transfer 4wks respectively. E&F.) Gastrocnemius muscle (mean ± SEM=1 ± 0.2228, n=3 and 1.904 ± 0.1162, n=6; t,df=4.034, 7; p=0.005 for sham and Foxp3$^{-/Y}$/Syt7$^{-/-}$ adoptive transfer 4wks respectively.



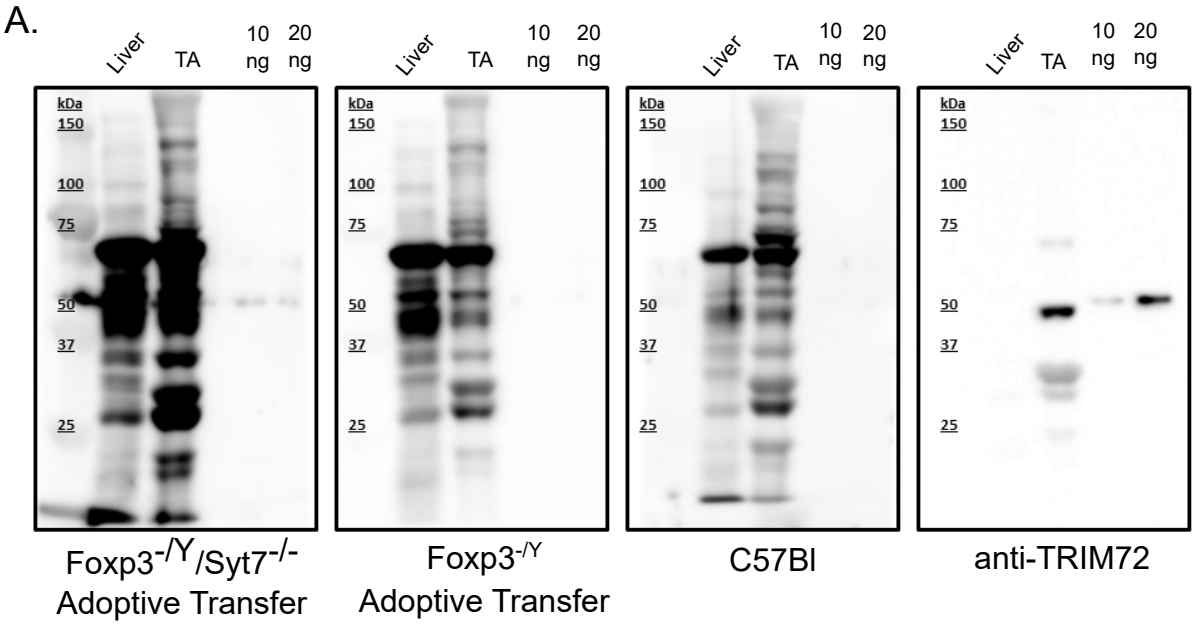
Supplemental Fig. 6. Sarcolemmal resealing capacity is significantly diminished following adoptive transfer of lymphocytes from Foxp3$^{-/Y}$/Syt7$^{-/-}$ mice 1 week post-adoptive transfer. Data from Fig. 4 is reproduced to highlight Sham 1wk versus adoptive transfer 1 wk (representative images of Sham 1wk are not included in Fig. 4.) Intact flexor digitorum brevis muscle bundles isolated from indicated mice and injured with an infrared laser in the presence of FM4-64 dye. The kinetics of sarcolemma resealing were measured by acquiring images every 3 seconds and calculating the change in fluorescence before and after injury over 60 seconds. A.) Representative images of Rag1$^{-/-}$ mice receiving either sham adoptive transfer or adoptive transfer of lymphocytes isolated from Foxp3$^{-/Y}$/Syt7$^{-/-}$ mice and FDB muscle fibers injured with an infrared laser. B.) Sarcolemmal resealing kinetics were measured every 3 seconds for 60 seconds. C.) Area under the curve calculations representing total dye influx over time. Statistical differences represent the ANOVA with Tukey HSD post-hoc analysis in Fig. 3F (n=27, 34 for sham 1wk and Foxp3$^{-/Y}$/Syt7$^{-/-}$ adoptive transfer respectively; $F(4,177)=28.47$, $p<0.0001$; Tukey HSD: sham vs Foxp3$^{-/Y}$/Syt7$^{-/-}$ 1wk $p<0.0001$).

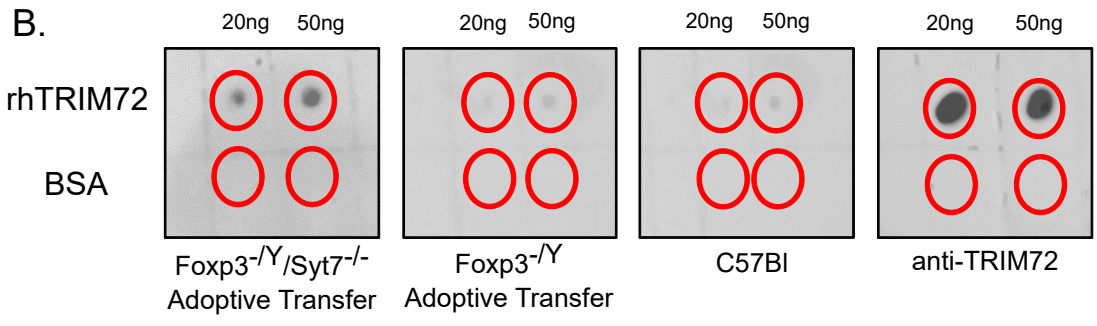


Supplemental Fig. 7. Tripartite motif protein levels in DM subject skeletal muscle biopsies. Skeletal muscle biopsies taken from healthy control subjects (Healthy), individuals diagnosed with SINM, or DM, screened for changes in TRIM family protein expression levels by western blot. A.) Representative western blots of indicated TRIM family proteins. B-H). Densitometry of TRIM family protein expression. B.) TRIM72: n=4, F(2, 9)=29.53, p=0.0001; Dunnett's Test: Healthy vs. SINM p=0.063; Healthy vs. DM p<0.0001. C) TRIM25: n=4; F(2, 9)=21.01, p=0.0004; Dunnett's Test: Healthy vs. SINM p=0.999; Healthy vs. DM p=0.0006. G.) TRIM27: n=4, F(2, 8)=7.387, p=0.015; Dunnett's Test: Healthy vs. SINM p=0.78, Healthy vs. DM p=0.012.

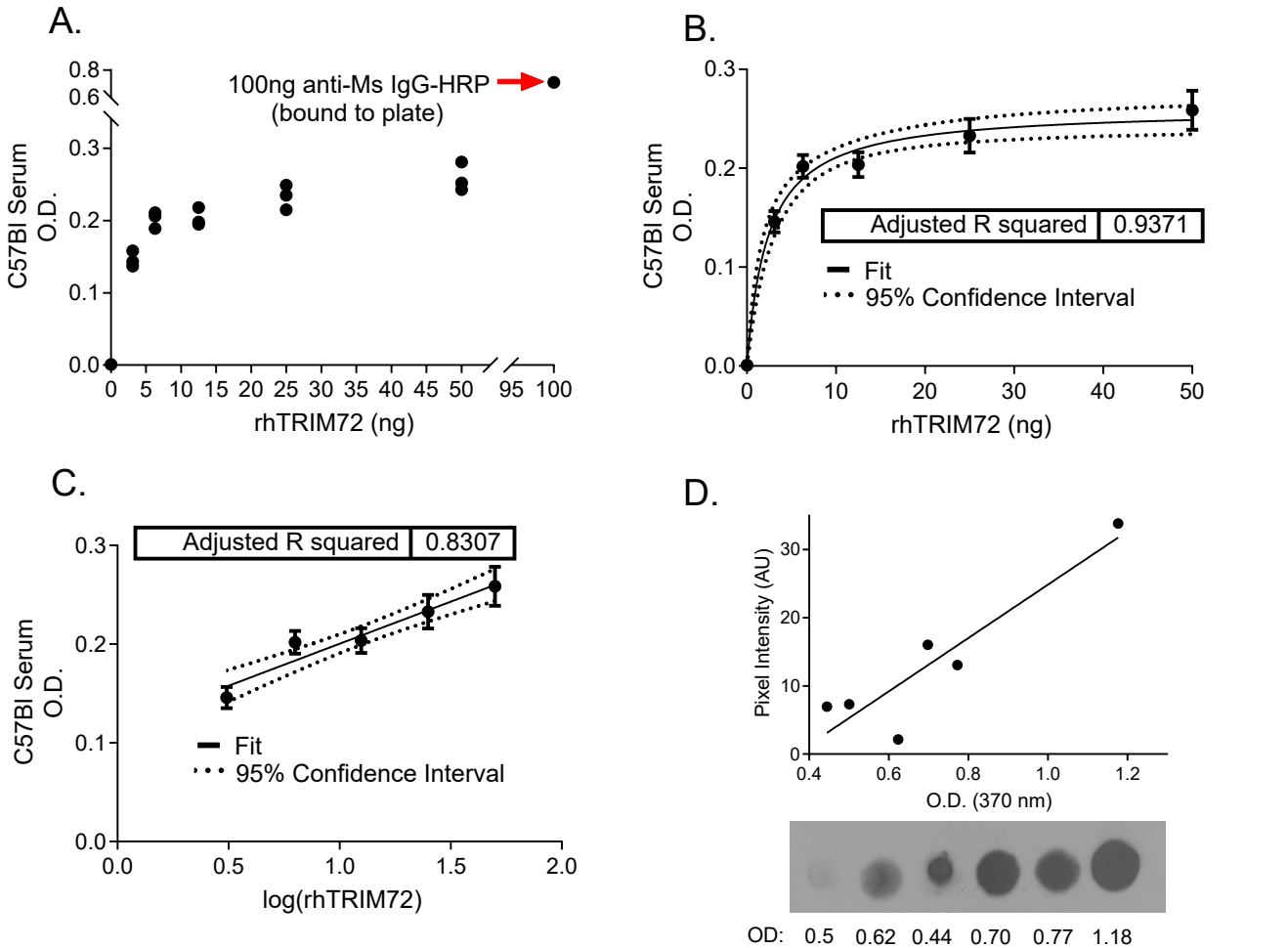


Supplemental Fig. 8. Preliminary identification of TRIM72 autoantibodies in IIM subject sera. HEK293 cells were transfected with TRIM family clones of interest. Each construct consisted of an individual TRIM family protein fused to eGFP. Expression levels were confirmed visually by fluorescent microscopy. Cell lysates were enriched for the TRIM protein of interest by immuno-pull down using anti-GFP antibody and protein AG conjugated agarose beads. Equal amounts of immuno-purified lysates were loaded onto a SDS-PAGE gel and separated by electrophoresis, transferred to nitrocellulose, and auto-antibodies against TRIM family proteins identified by western blot using IIM subject serum as the primary antibody. Representative images of western blots identifying various TRIM family proteins as potential autoantigens associated with IIM in two subject sera samples (A and B). Arrowhead indicates band of interest. Molecular weight of TRIM72 is shifted due to the GFP-fusion construct. Additional TRIM family proteins tentatively identified using this method as potential IIM autoantigens not shown: TRIM10, TRIM27, TRIM63/MURF1, TRIM38, and TRIM58

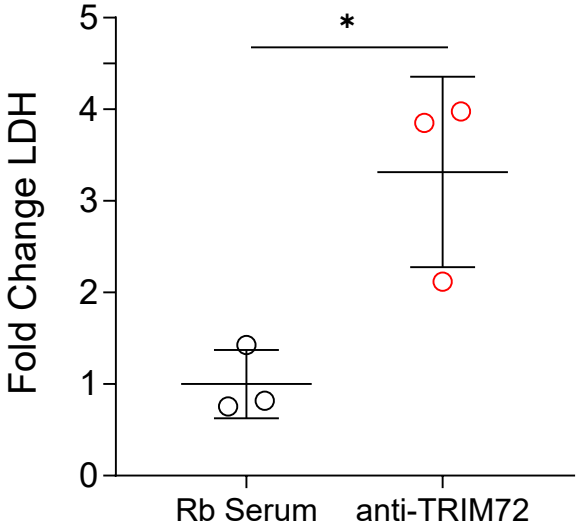




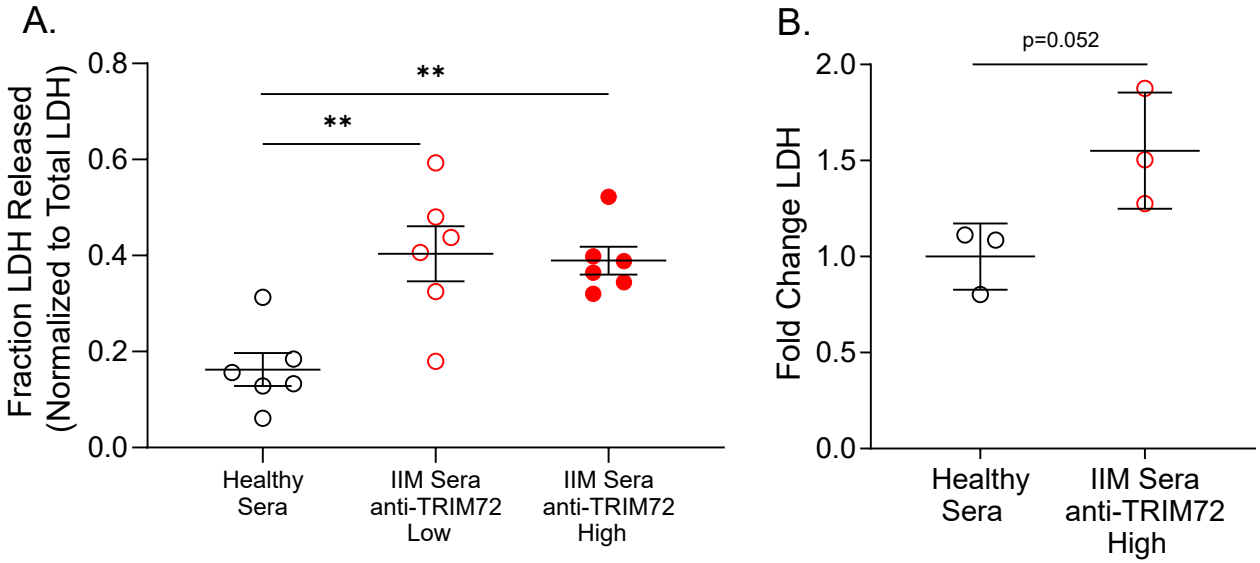
Supplemental Fig. 9. Autoantibodies targeting TRIM72 are present in an adoptive transfer model of myositis. A.) Liver homogenate (negative for TRIM72), tibialis anterior (positive for TRIM72), and recombinant human TRIM72 protein (10 and 20ng) separated by SDS-Page and transferred to nitrocellulose membranes. Incubation with a 1:200 dilution of serum collected from indicated mice as the primary antibody identified TRIM72 as a potential autoantigen. B.) Verification of results represented in A. by dot blot. 20 and 50ng rhTRIM72 protein dotted onto nitrocellulose and incubated with indicated serum collected from mice at a dilution of 1:100.



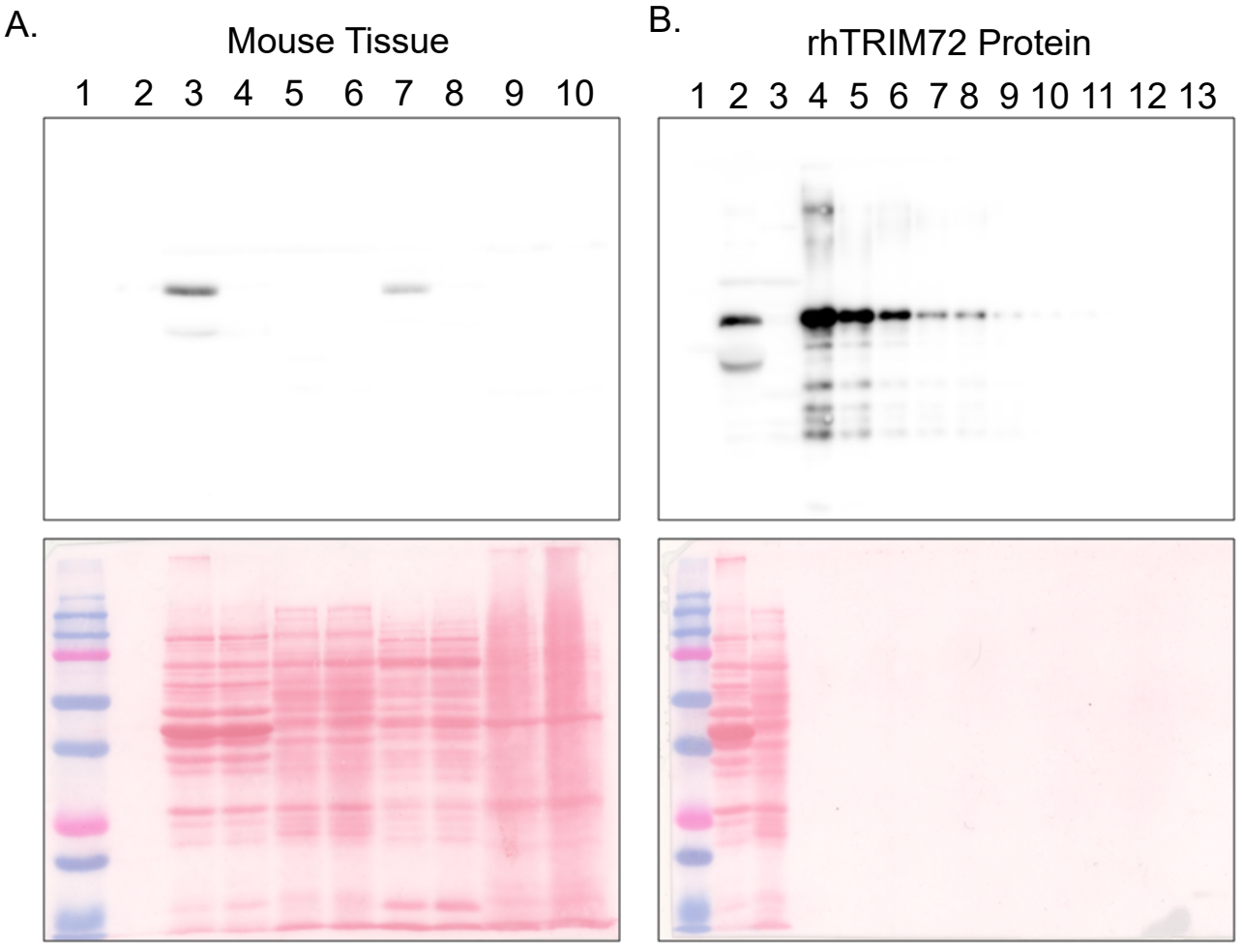
Supplemental Fig. 10. Optimization of custom ELISA to detect autoantibodies against TRIM72 within the saturation limits of TMB substrate and to provide sufficient linear range to account for nonspecific IgG binding. Solid line = fitted equation, dotted line = 95% confidence interval, O.D. saturation limit of TMB substrate = 2.0. Serial concentrations of recombinant human TRIM72 protein ranging from 0ng to 100ng were coated on an ELISA plates in triplicate overnight. 100ng of polyclonal anti-mouse IgG coated in wells was used to determine a saturating limit for the enzymatic reaction. Serum collected from C57Bl mice diluted 1:50 was added to each well and incubated for 2 hours at room temperature. A.) Raw O.D. values. B.) Exponential fit of mean values and adjusted R squared value. Background levels of nonspecific IgG in healthy C57Bl control serum samples plateau at approximate O.D. readings of 0.26 corresponding to 50ng rhTRIM72 and are 2-fold lower than 100% IgG-HRP O.D. levels. C.) Log fit of rhTRIM72 concentration. D.) Correlation of dot blot pixel intensity obtained from IIM patient serum samples to O.D. values obtained by ELISA.



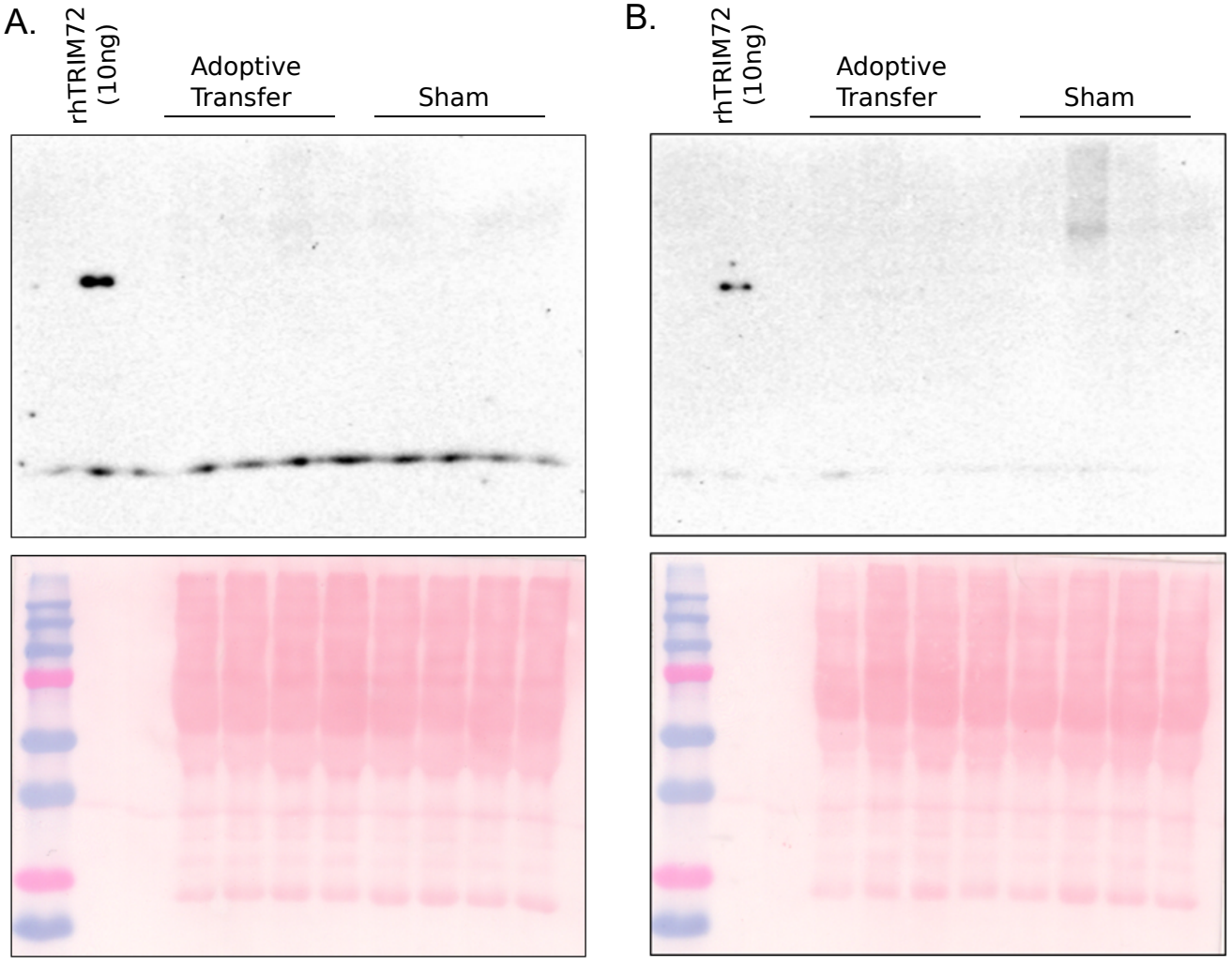
Supplemental Fig. 11. Tertiary validation of exogenous delivered polyclonal antibody against TRIM72 is sufficient to reduce membrane resealing capacity. C2C12 myoblasts allowed to reach approximately 70% confluence and stimulated to differentiate into multinucleated myotubes (complete DMEM supplemented with 2% horse serum) injured with 600μm glass beads on an orbital shaker for 1 minute with normal rabbit serum (control) or exogenously delivered anti-TRIM72 polyclonal antibody. LDH release was quantified as a measure of membrane resealing capacity. n=3, t,df=3.632, 4; p=0.0221.



Supplemental Fig. 12. Secondary and tertiary validation of IIM subject sera with elevated TRIM72 autoantibody levels negatively affect sarcolemmal resealing capacity. A.) HEK293 cells transfected to express TRIM72-GFP were injured using a rotational damage assay in the presence of serum from healthy subjects and IIM subjects with low (O.D.>1 SD) or high (O.D.>2 SD) levels of anti-TRIM72 as previously determined by ELISA. n=6; $F(2,15)=10.31$; $p=0.0015$; Tukey's HSD: Healthy vs. Low $p=0.0029$, Healthy vs. High $p=0.0046$. B.) C2C12 myoblasts stimulated to differentiate into multinucleated myotubes then injured with gold beads by ballistic assay described in Methods. n=3; t, $df=2.742, 4$; $p=0.0518$.



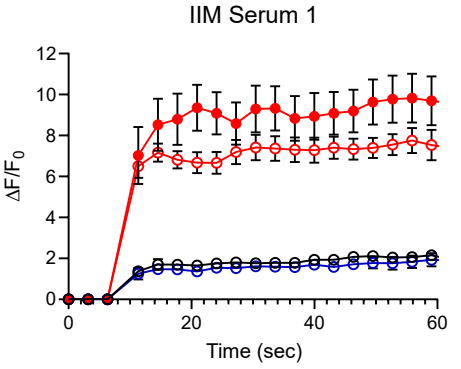
Supplemental Fig. 13. Specificity of polyclonal anti-TRIM72 raised in rabbit. A.) Western blot of tissues collected from C57Bl and TRIM72$^{-/-}$ mice. Lanes: 1) Marker, 2) 10 ng rhTRIM72, 3) C57Bl skeletal muscle, 4) TRIM72$^{-/-}$ skeletal muscle, 5) C57Bl liver, 6) TRIM72-/- liver, 7) C57Bl heart, 8) TRIM72-/- heart, 9) C57Bl lymphocytes, 10) TRIM72$^{-/-}$ lymphocytes. B.) Decreasing dilutions of rhTRIM72 protein. Lanes: 1) Marker, 2) C57Bl skeletal muscle, 3) C57Bl liver, 4) 100 ng rhTRIM72, 5) 50 ng, 6) 25 ng, 7) 15 ng, 8) 10 ng, 9) 5 ng, 10) 2 ng, 11) 1 ng, 12) 0.5 ng, 13) 0.25 ng.

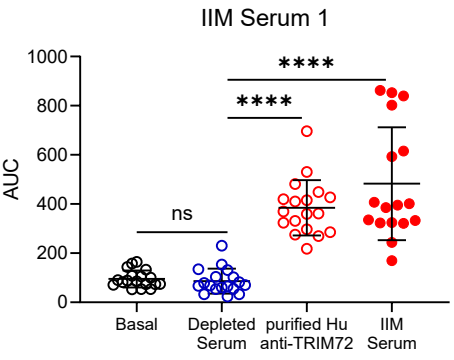


Supplemental Fig. 14. TRIM72 is not detected in sera from $Rag1^{-/-}$ mice receiving adoptive transfer of lymphocytes from $Foxp3^{-/Y}/Syt7^{-/-}$ mice. 3μL of sera isolated from 1 week post-adoptive transfer mice (A.) and 4 weeks post-adoptive transfer mice (B.) were separated by SDS-Page and western blots performed to detect circulating levels of TRIM72 protein. rhTRIM72 protein was used as a positive control.

A.

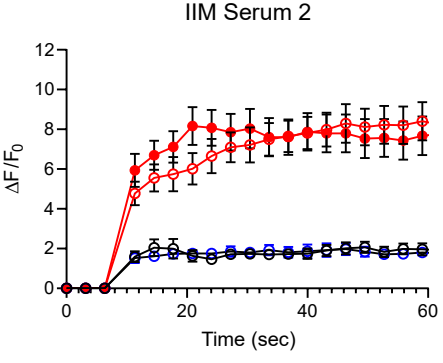
IIM Serum 1	
A280	0.467
Eluted IgG (μg/mL)	127.3

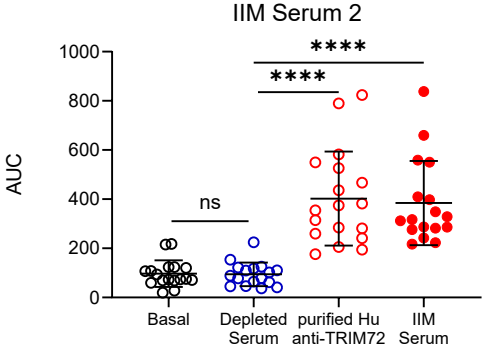




B.

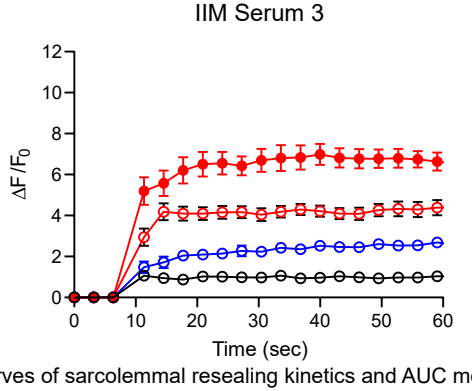
IIM Serum 2	
A280	0.496
Eluted IgG (μg/mL)	147.2

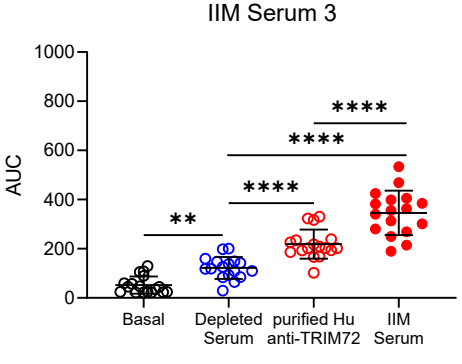




C.

IIM Serum 3	
A280	0..84
Eluted IgG (μg/mL)	251.3





Supplemental Fig. 15. Curves of sarcolemmal resealing kinetics and AUC measurements of total dye influx over time from individual IIM patient serum depleted of TRIM72 autoantibodies depicted in Fig. 8. Tables indicate A280 values and concentration of eluted IgG. A.) n=18, 18, 17, and 18 for Basal, Depleted Serum , IIM Serum, and Purified Human anti-TRIM72 respectively. B.) n=17, 17, 17, and 18. C.) n=17, 17, 17, and 17. ** p<0.01, **** p<0.0001.