

Liver and Pancreatic-Targeted Interleukin-22 as a Therapeutic for Metabolic Dysfunction-Associated Steatohepatitis

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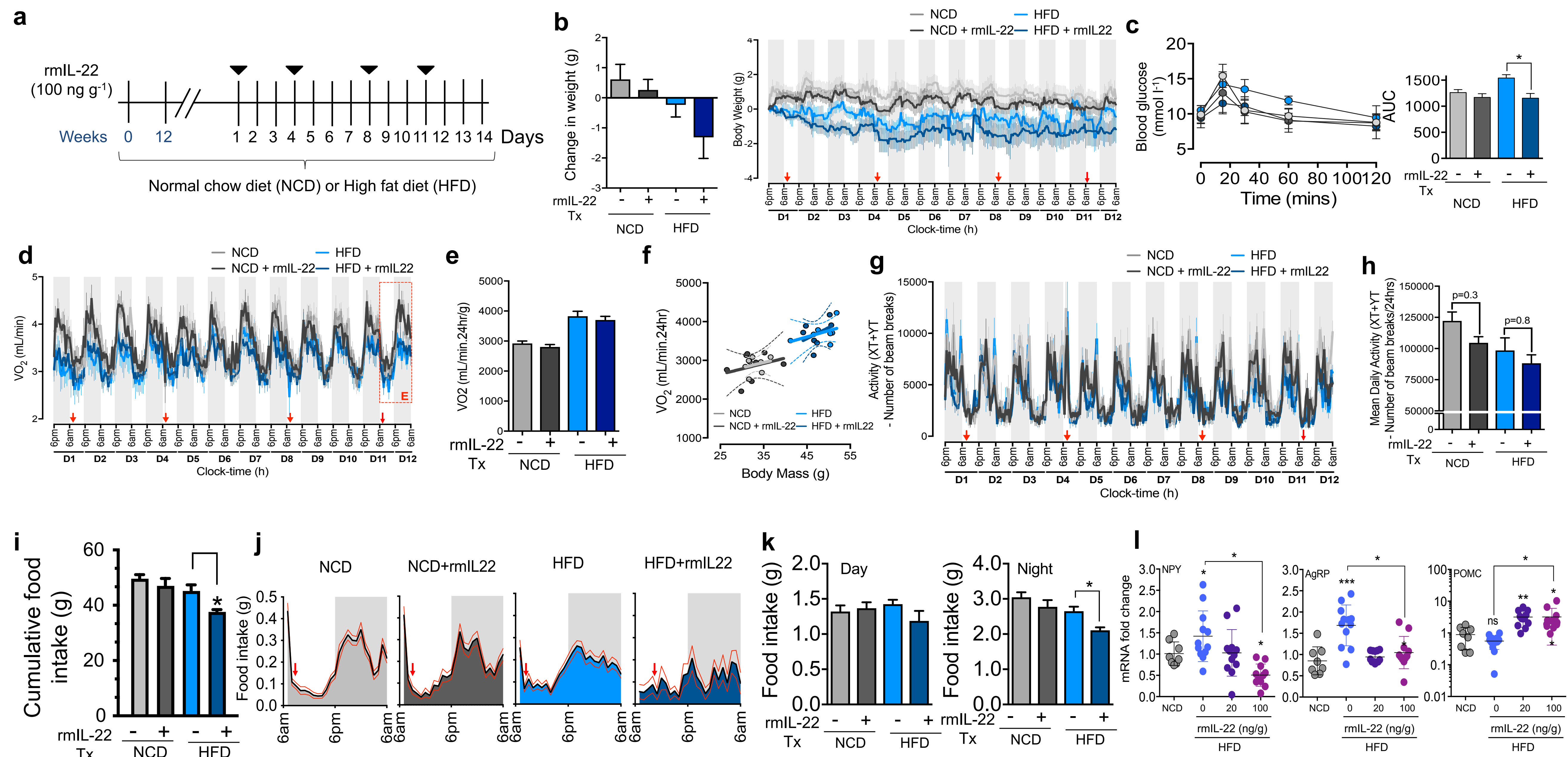
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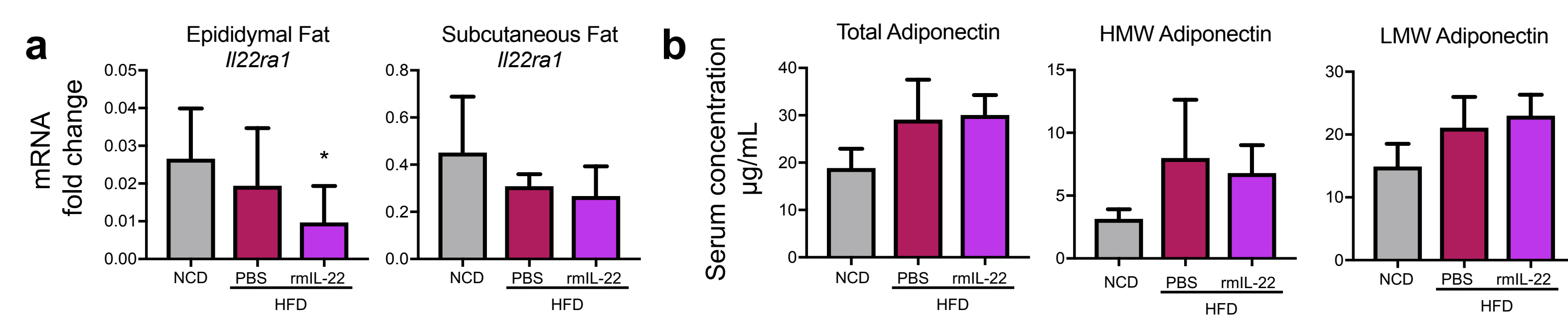
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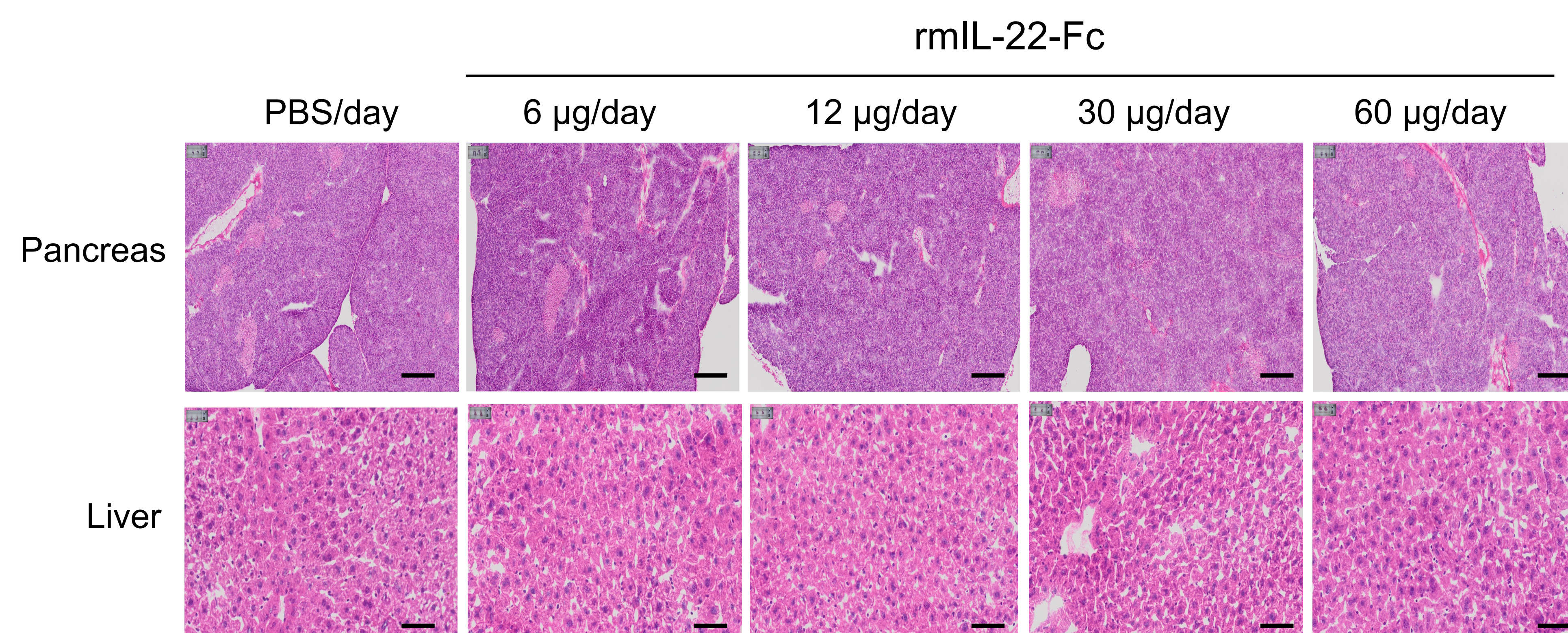
Supplementary Information



Supplementary Figure 1: Recombinant mouse IL-22 improved glucose tolerance without altering metabolic rate or energy expenditure and may influence satiety. (a) Schematic showing mice were fed a normal chow diet (NCD) or a high fat diet (HFD) for 14 weeks and treated biweekly for the last two weeks with 100 ng g⁻¹ of recombinant mouse IL-22 (rmIL-22), i.p. Control NCD and HFD animals received PBS. (b) decrease in body weight of HFD animals treated with rmIL-22, no changes in NCD animals treated with rmIL-22. Average real-time measurements of body weight across the first 12 days of treatment recorded using TSE phenomaster system. Red arrows depict the rmIL-22 treatments. (c) Oral glucose tolerance test (oGTT) and area under the curve (AUC) shows significant improvements in hyperglycaemia with rmIL-22 treatment. No alterations in the (d) average VO₂ measurements (mL min⁻¹), (e) VO₂ on day 11, (f) or VO₂ when compared to body mass of the animals; ANCOVA was used to adjust for the influence of body weight. (g) Average real time activity and (h) activity of animals in the last 24 h as determined through beam breaks shows no differences between treated and untreated animals. (i) Change in cumulative food intake over the experimental period demonstrates the decrease in total food consumption in HFD animals treated with rmIL-22. Not seen in NCD animals treated with rmIL-22. (j) Food intake measurements on day 11 (average block colours, +/- SD in red) shows the reduced intake of HFD animals treated with rmIL-22 (k) shown that changes in food intake occur during the night only in the HFD animals treated with rmIL-22. (l) Gene expression changes in neuronal subpopulations. a-k: n=7-8; m: n = 8-12 ANOVA, Bonferroni's *post hoc* test. *p<0.05; ***p<0.001. Source data are provided as a Source Data file



Supplementary Figure 2: No change in adiponectin with rmIL-22 treatment. Mice were fed a normal chow diet (NCD) or a high fat diet (HFD) for 14 weeks and treated biweekly for the last two weeks with 100 ng g⁻¹ of recombinant mouse IL-22 (rmIL-22), i.p. Control NCD and HFD animals received PBS. Experimental schematic shown in Figure 1a. mRNA expression analyses for **(a)** IL-22 receptor (*Il22ra1*) **(b)** No significant changes were observed in the circulating serum levels of total, high molecular weight (HMW) or low molecular weight (LMW) adiponectin protein. n=8, ANOVA, Bonferroni's *post hoc* test. *p<0.05 compared to NCD mice. Source data are provided as a Source Data file



Supplementary Figure 3: No major changes in pancreas and liver with chronic administration of long-circulating rmIL-22-Fc. C57BL/6 animals were treated with either PBS or 6, 12, 30 or 60 μ g of rmIL-22-Fc daily for 8 weeks (s.c.). Schematic shown in Figure 1f. H&E staining of pancreas (scale bar: 100 μ m) and liver (scale bar: 50 μ m).

a

hIL-22

MGWSCIIILFLVATATGVHSL EEQKLISEEDLNAPIS SHCRLDKSNFQQPYITNRTFMLAKEASLADNNTDVRLIGEKL FHGVSMSERCYLMKQVLNFTLEEVLFPQSDRFQPYMQE VVPFLARLSNRLSTCHIEGDDLHIQRNVQKLKDTVKKLGESGEIKAIGELDLLFMSLRNACIHHHHHHH-

ScFv-hIL-22

MGWSCIIILFLVATATGVHSEL EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSITAEGTHTWYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKTSYRFDYWGGGLTVTVSSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYKASRLQSGVPSRFSGSGSGTDFTLTITSS LQPEDFATYYCQQKWDP PRTFGQGTKVEIKRSGGGGSAPIS SHCRLDKSNFQQPYITNRTFMLAKEASLADNNTDVRLIGEKL FHGVSMSERCYLMKQVLNFTLEEVLFPQSDRFQPYMQE VVPFLARLSNRLSTCHIEGDDLHIQRNVQKLKDTVKKLGESGEIKAIGELDLLFMSLRNACIEQKLISEEDLNHHHHHHH-

hIL-22-ScFv

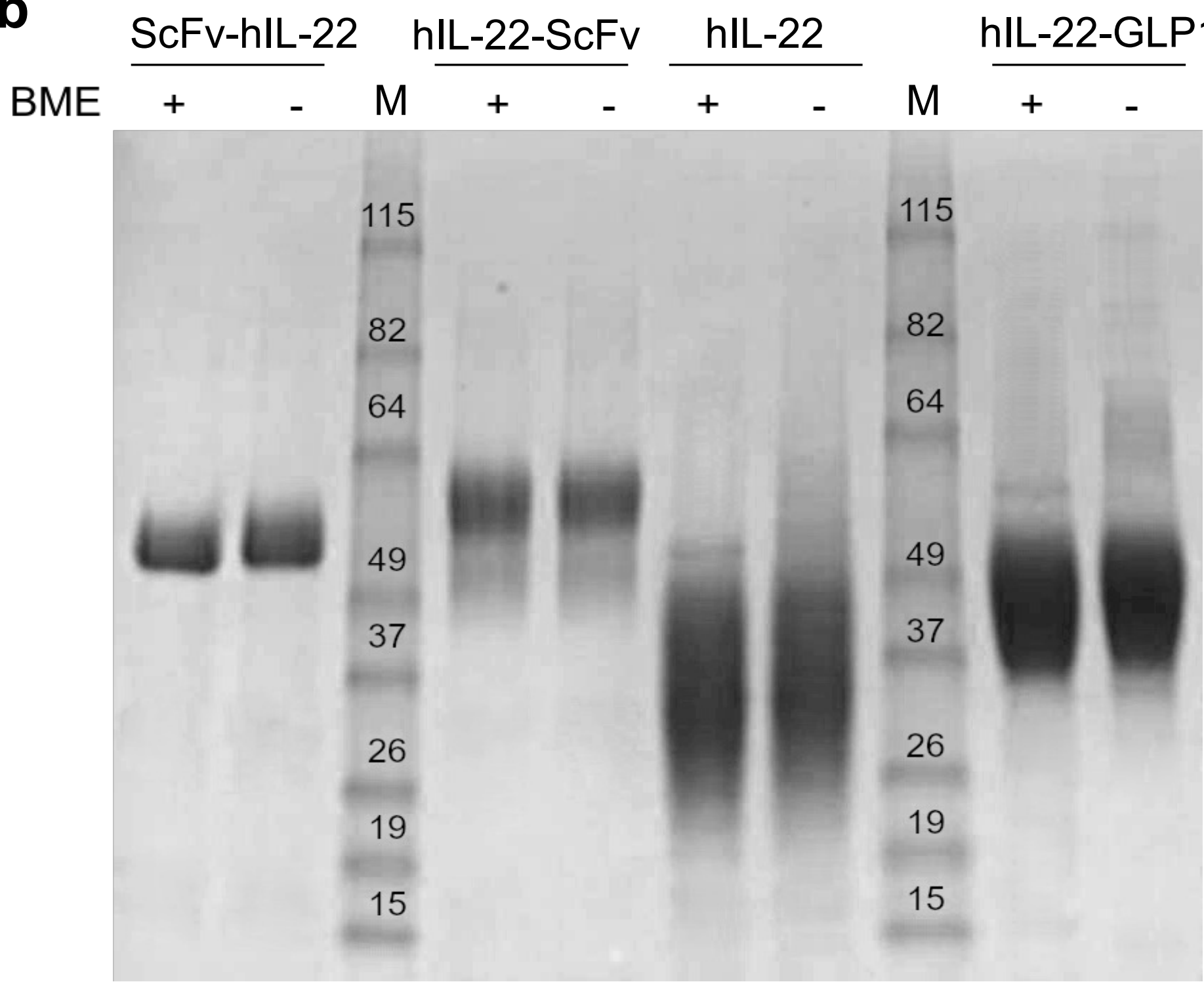
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hIL-22-GLP1

MGWSCIIILFLVATATGVHSHGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPSGSSSSGGGSSSSGGGSSSSGGGSSSGAPIS SHCRLDKSNFQQPYITNRTFMLAKEASLADNNTDVRLIGEKL FHGVSMSERCYLMKQVLNFTLEEVLFPQSDRFQPYMQE VVPFLARLSNRLSTCHIEGDDLHIQRNVQKLKDTVKKLGESGEIKAIGELDLLFMSLRNACIGGSHHHHHHHHGGSEQKLISEEDL-

Key: Leader sequence; ScFv; human IL-22; GLP1 (Exe-4)

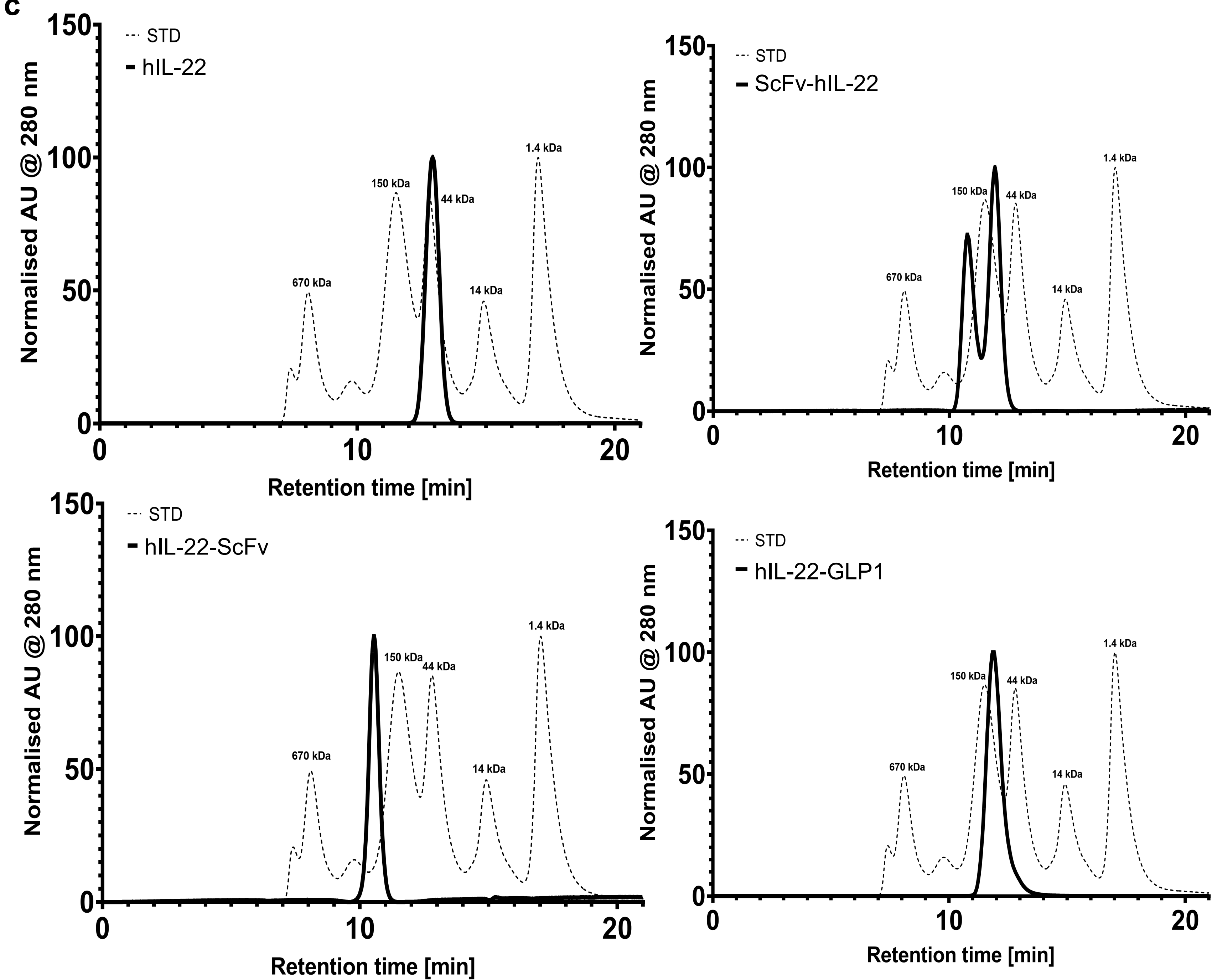
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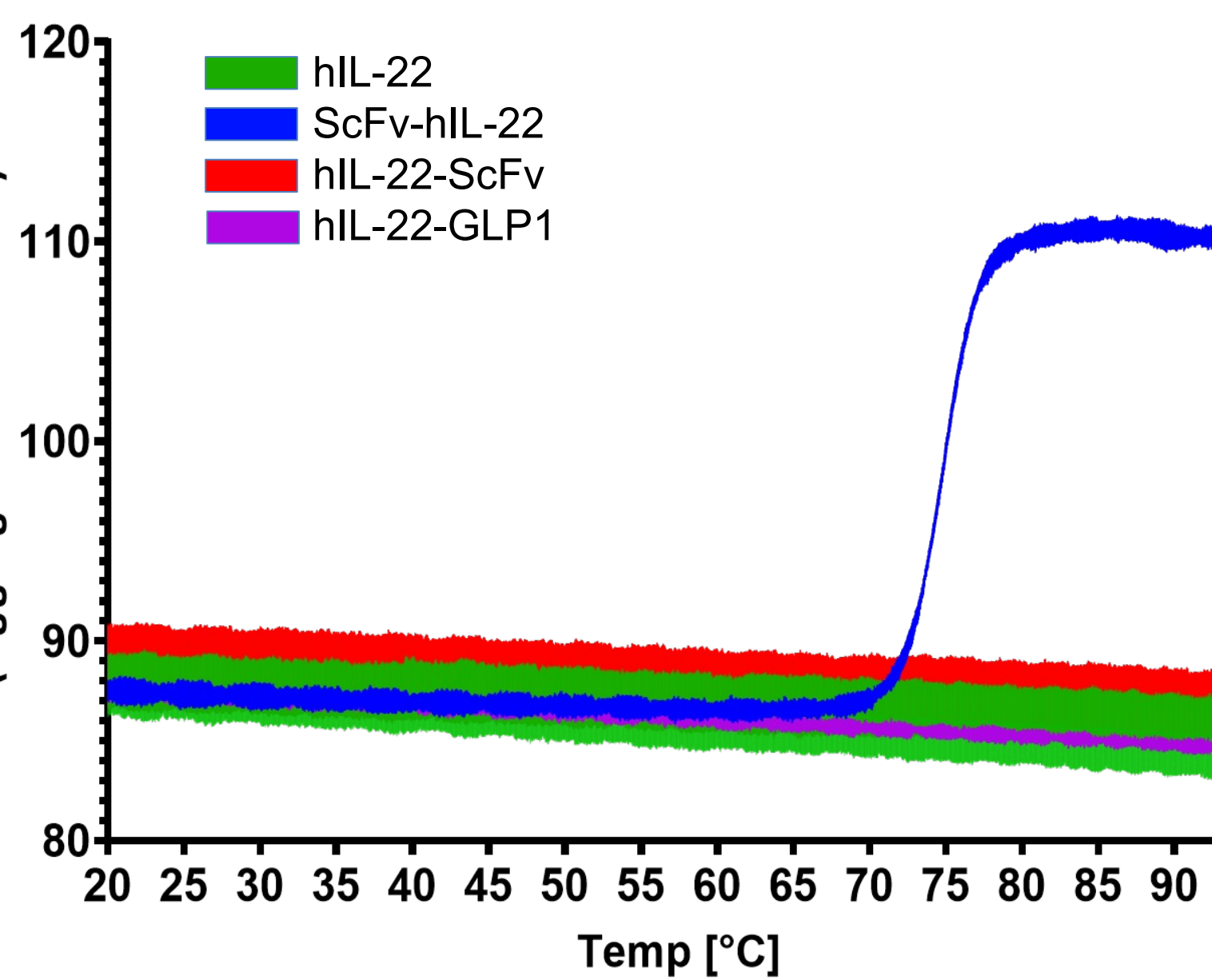
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Clone	Apparent Mw [kDa]	Oligomer
hIL-22	37	2
ScFv-hIL-22	162 / 73	3 / 1
hIL-22-ScFv	187	4
hIL-22-GLP1	76	2

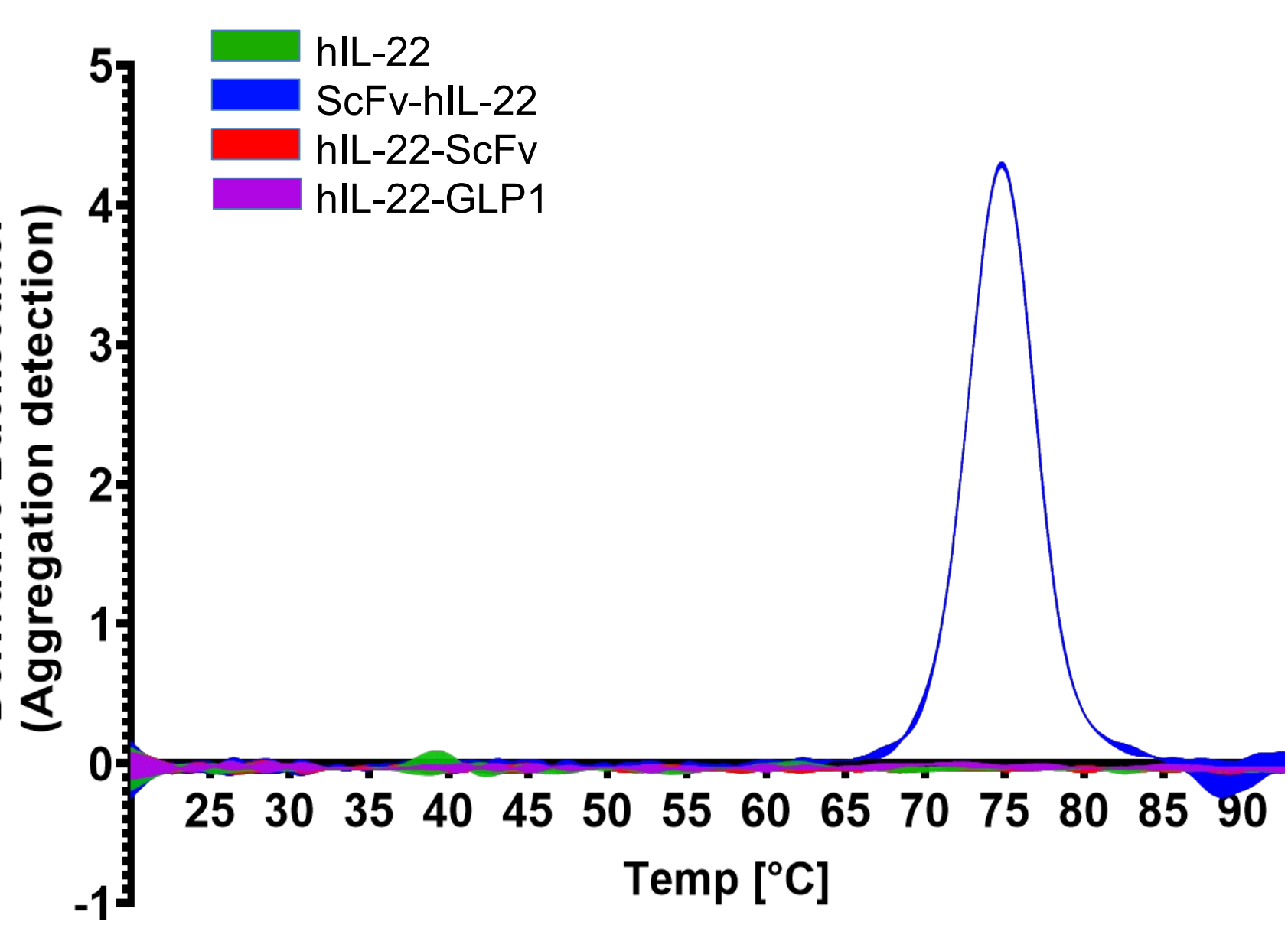
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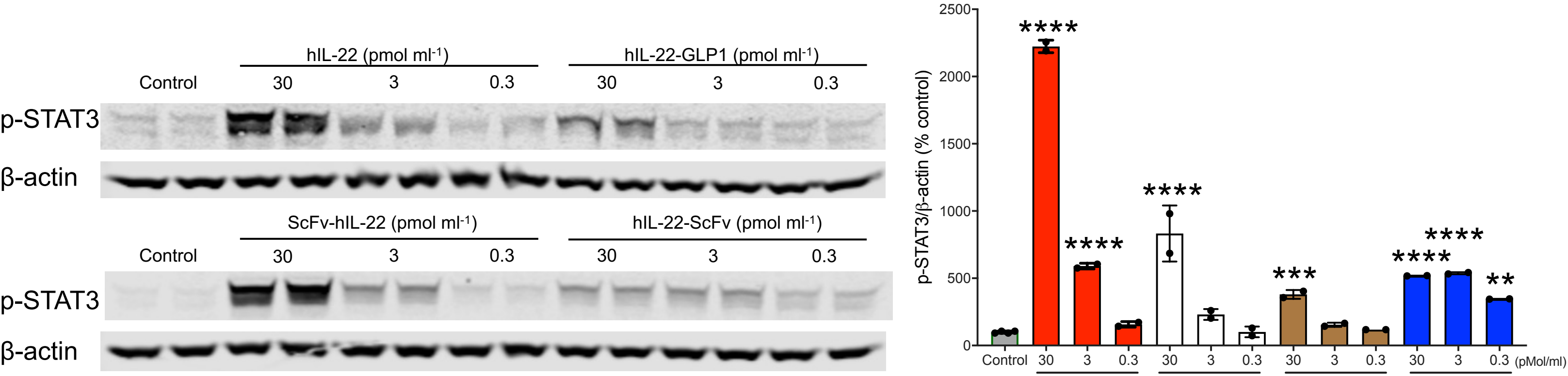


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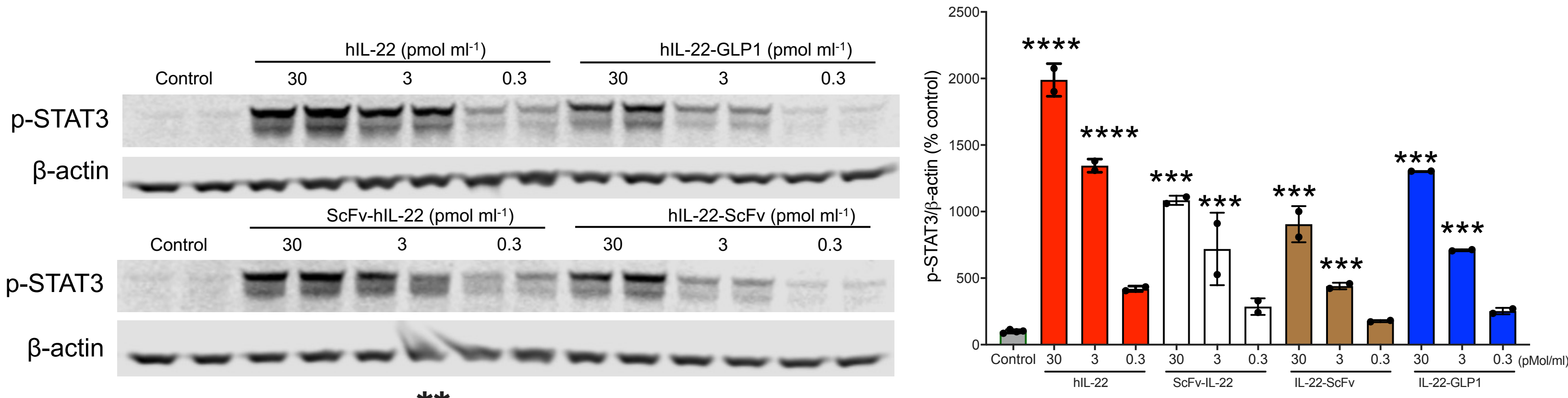


Supplementary Figure 4: Human IL-22-fusion proteins. (a) All proteins contained N-terminus Myc and a C-terminus His-tag. hIL-22-fusions contained linkers as shown in sequences above. (b) Purified proteins were analysed using western blotting under reducing (+BME) and non-reducing conditions (-BME). Lane ‘M’ shows the marker in kDa. (c) Analytical HPLC SEC aggregation analyses of purified proteins shows potential aggregation of ScFv-hIL-22. The apparent molecular weight (Mw) is shown in (d). (e) Aggregation and (f) aggregation derivative shows that ScFv-hIL-22 aggregates at higher temperatures and therefore may have low stability in-vivo. Source data are provided as a Source Data file

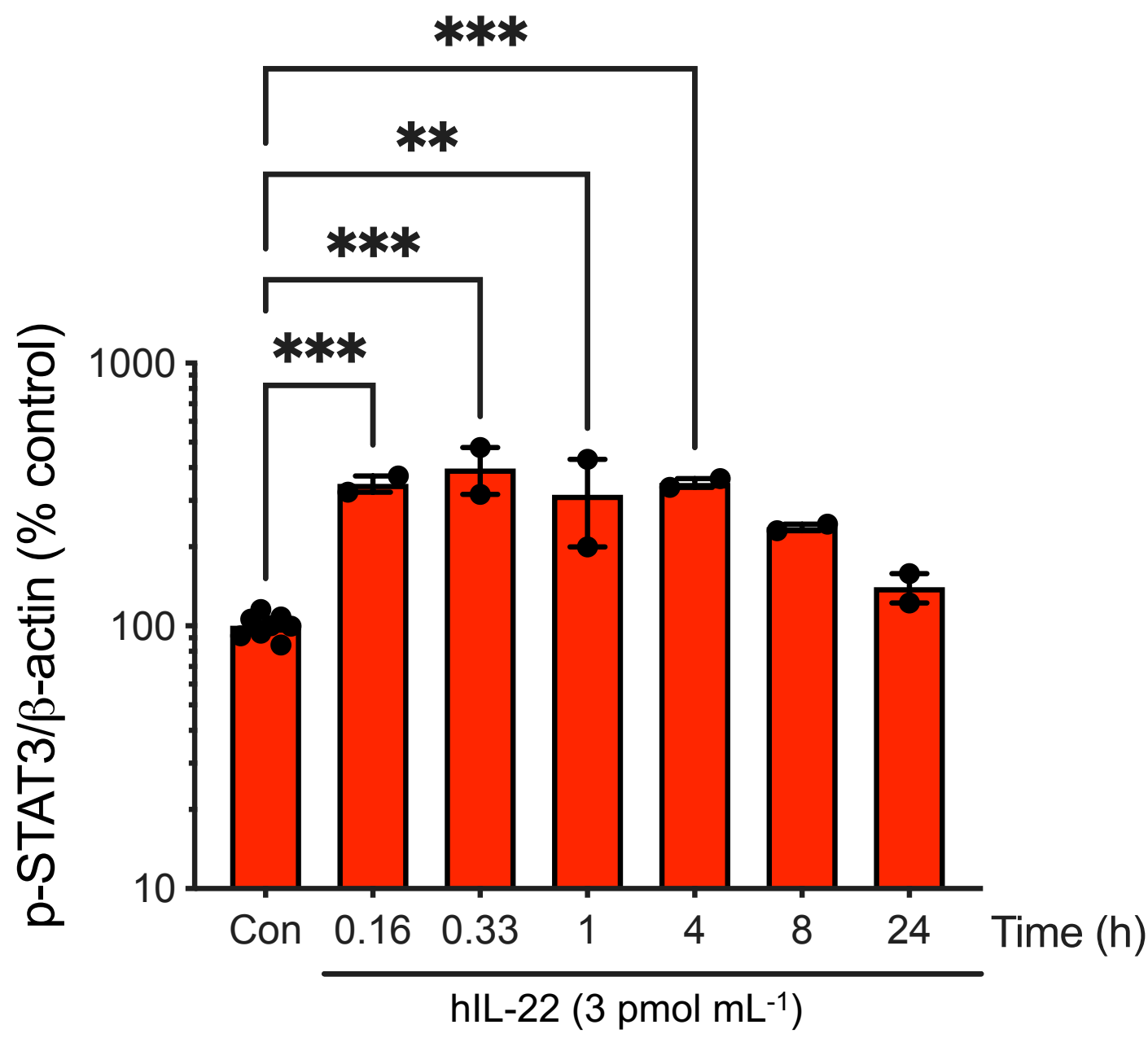
a MIN6N8 cells



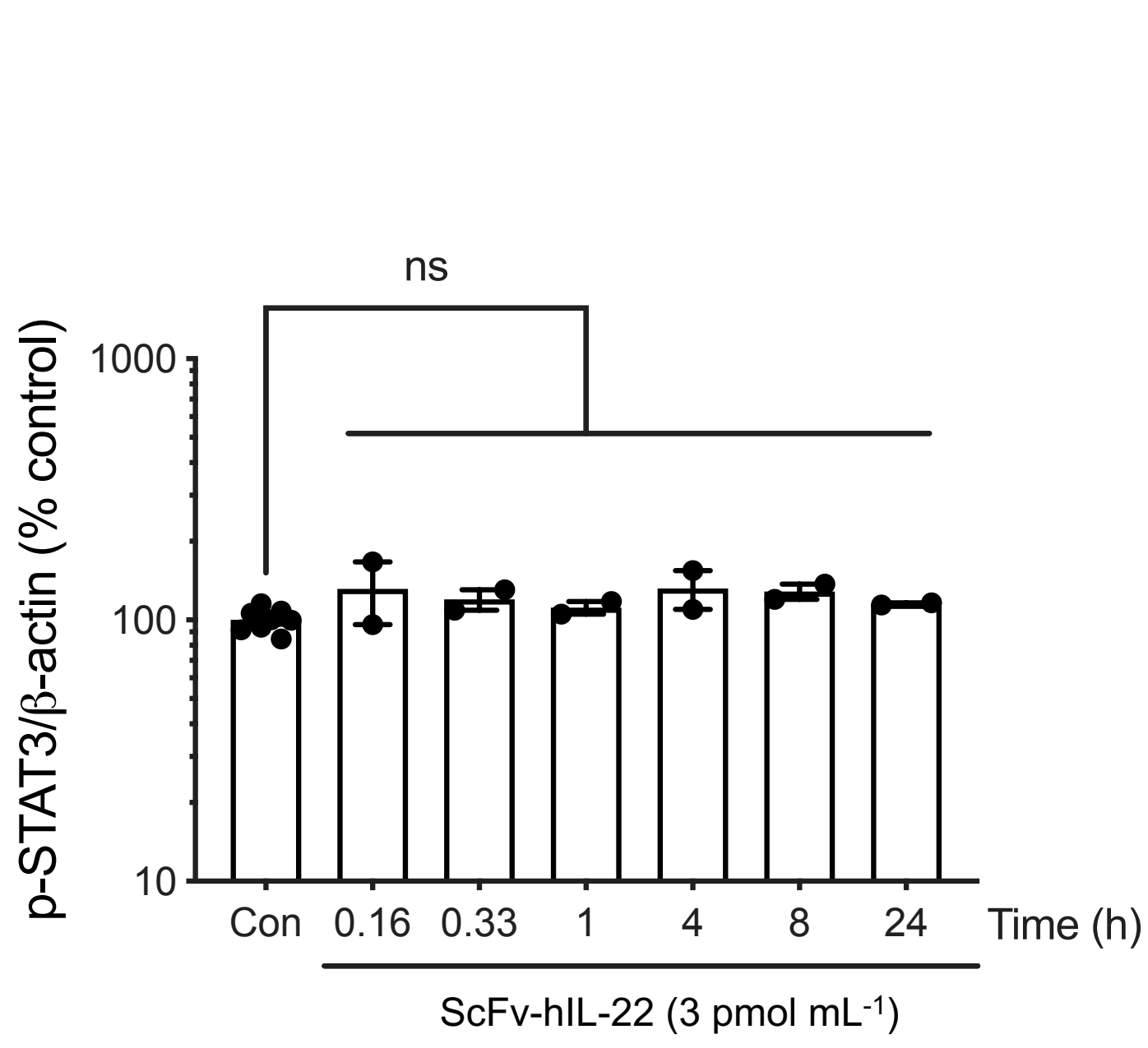
b HEPG2 cells



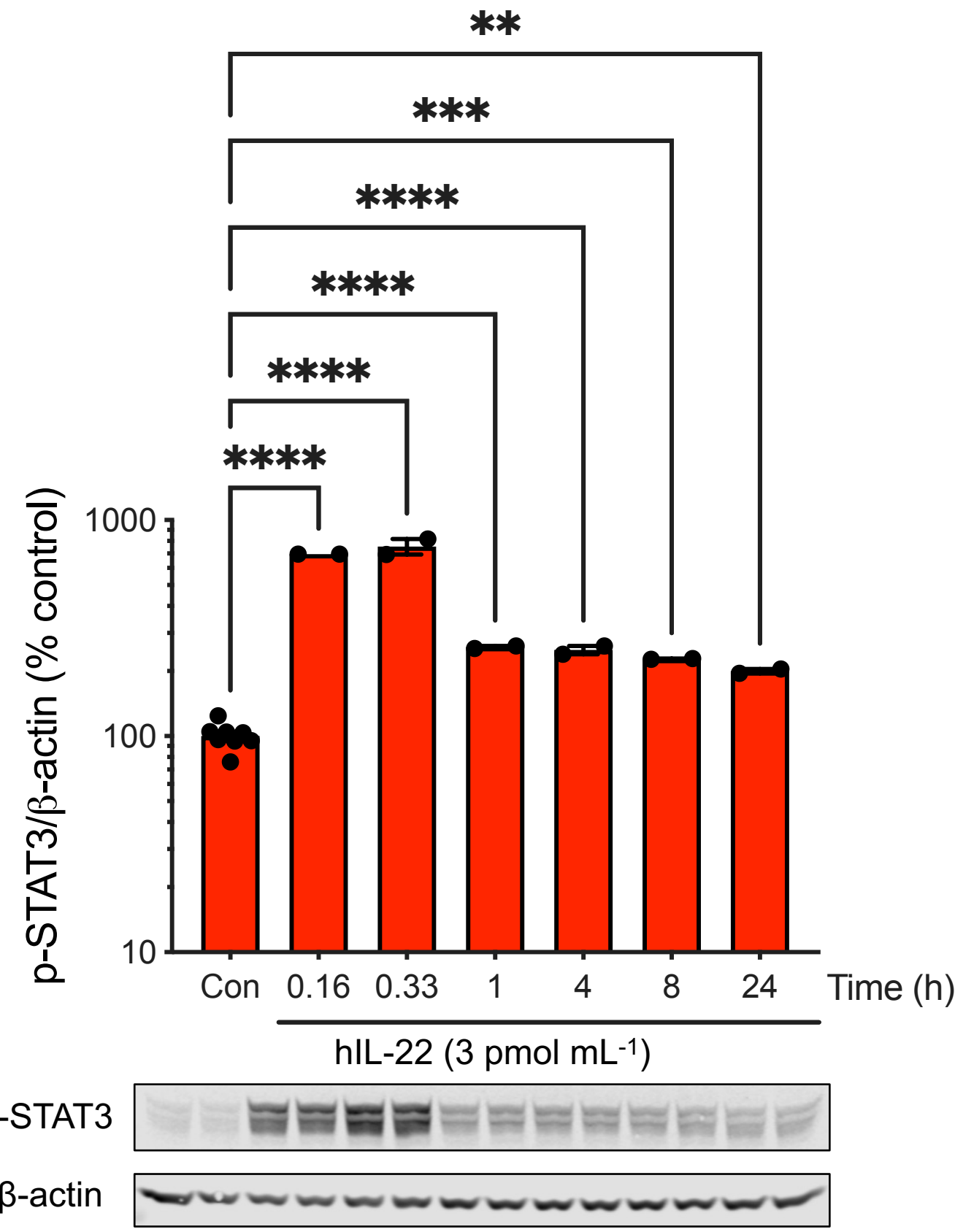
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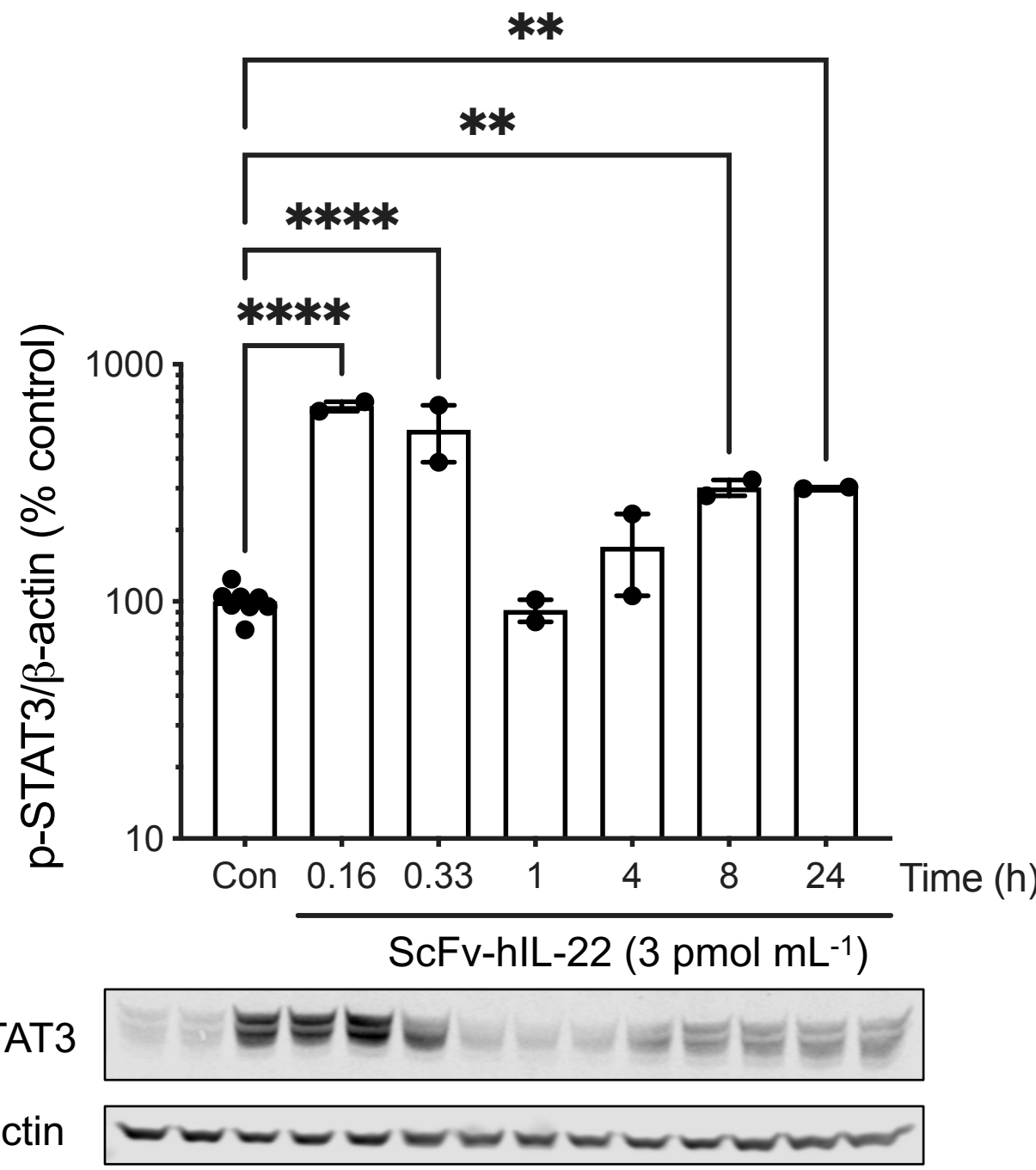
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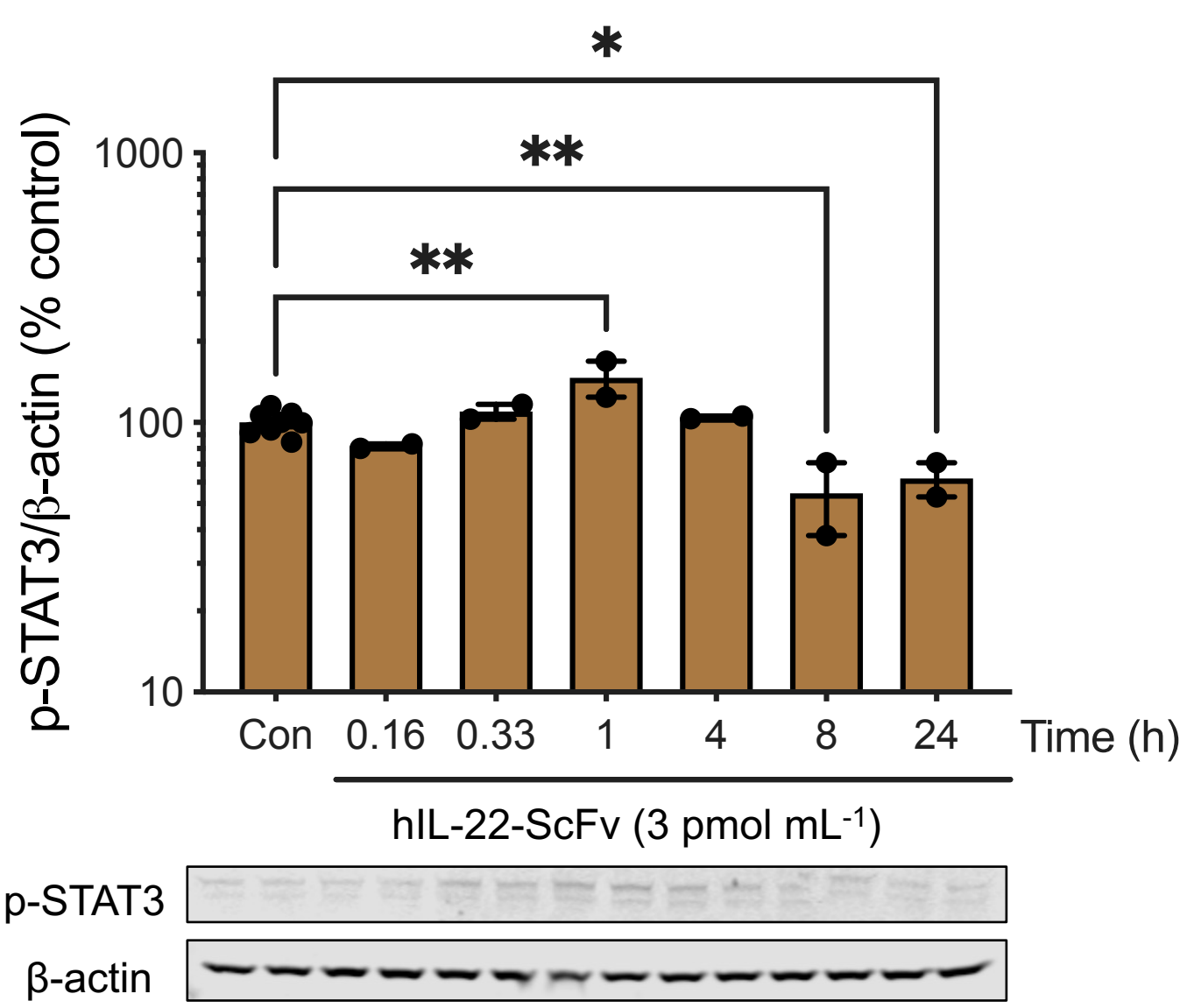
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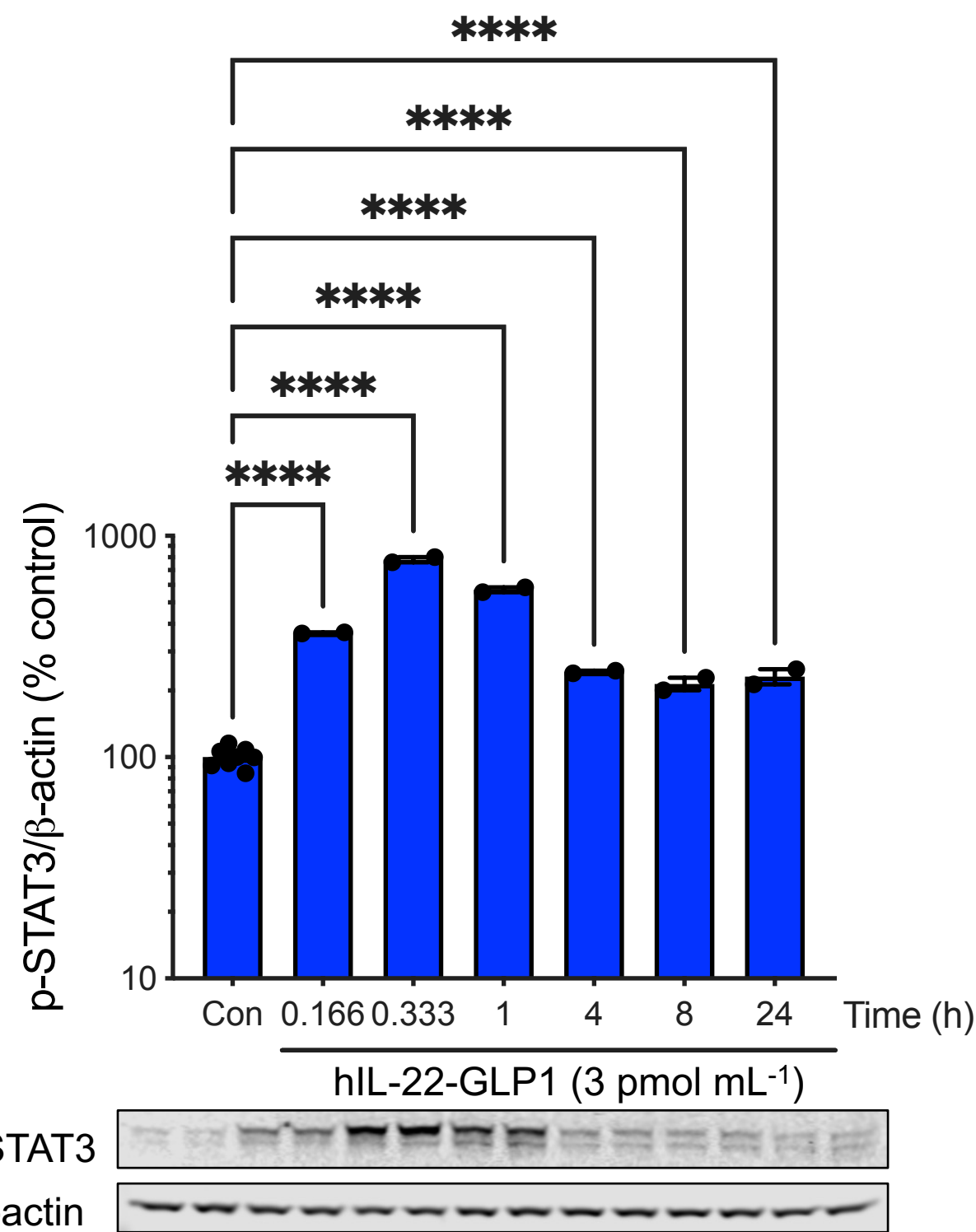
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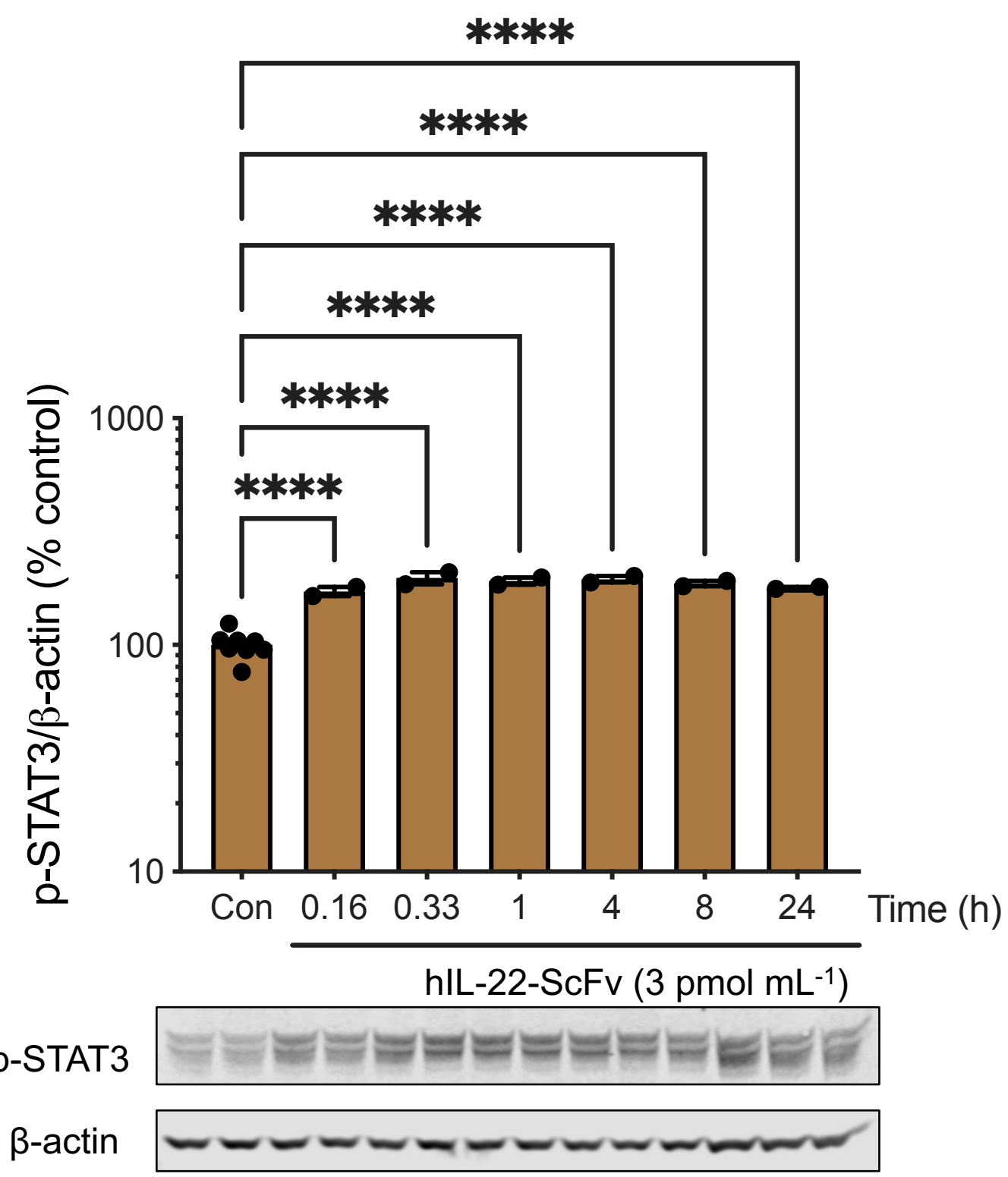
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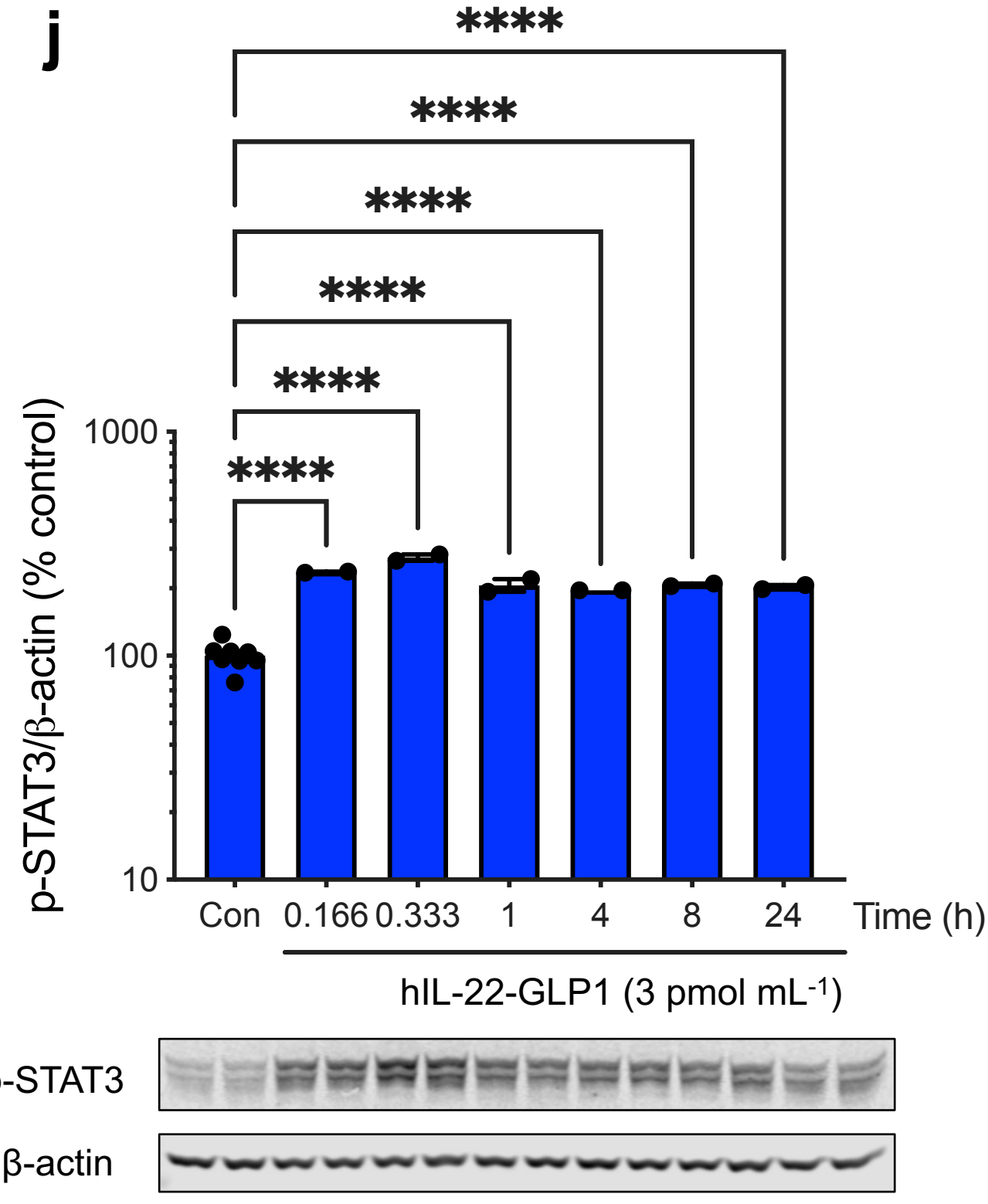
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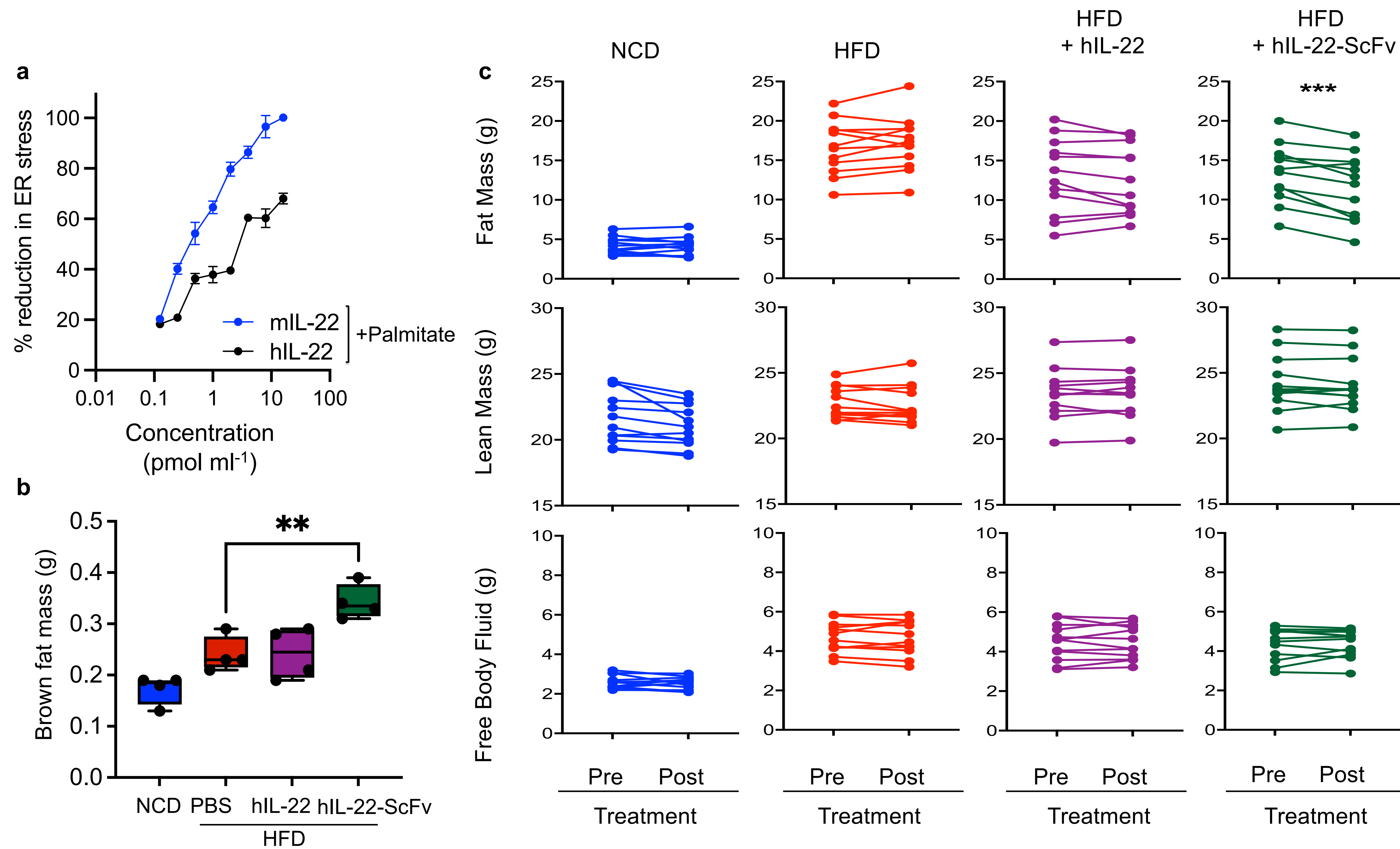
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Supplementary Figure 5: Human IL-22-fusion proteins activate STAT3p in MIN6N8 and HEPG2 cell lines. (a) MIN6N8 and (b) HEPG2 cells were treated with hIL-22, ScFv-hIL-22, hIL-22-ScFv and hIL-22-GLP1 at 30, 3 and 0.3 pmol mL⁻¹ concentration for 30 mins, protein was isolated and analysed using western blotting for STAT3-phosphorylation. (c-f) MIN6N8 cells and (g-j) HEPG2 cells were stimulated with native hIL-22 and hIL-22 fusions were used at 3 pmol mL⁻¹ concentration and STAT3p was assessed at 6 different time points (0.16, 0.33, 1, 4, 8, 24 h) to assess the kinetics of activation. Representative data from 2 independent experiments. PBS was used a vehicle control (con) for all experiments. ANOVA, Bonferroni's post hoc test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to control. Source data are provided as a Source Data file

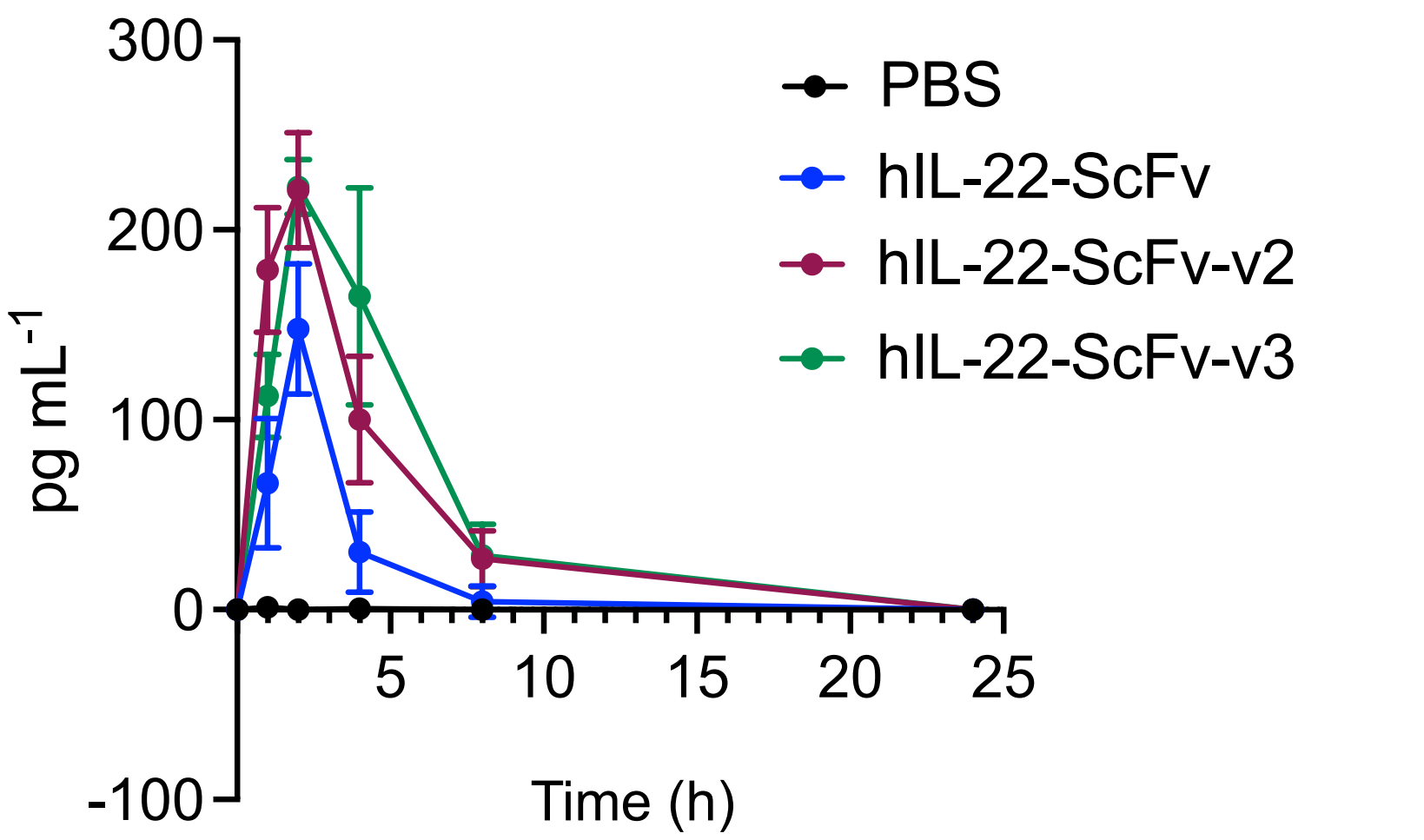


Supplementary Figure 6: Efficacy of hIL-22 and targeted hIL-22. (a) MIN6N8 cells transfected with the ERAI reporter were treated with 0.2 mM palmitate for 24 h prior to treatment with increasing doses of recombinant mouse IL-22 (mIL-22) or human IL-22 (hIL-22) at increasing concentrations (0.125 – 16 pmol mL⁻¹). Data presented as the percentage reduction from the palmitate only. n = 4. (b) Brown fat (c) and Nuclear magnetic resonance (NMR) was used to determine the changes in fat mass of animals intraperitoneally treated with hIL-22 or hIL-22-ScFv (schematic shown in Fig. 3a). Fat mass, lean mass and free body fluids is shown in grams pre and post treatment. (b) One-way ANOVA, non-parametric; *P<0.01 compared to HDS-PBS control. (c) Paired t-test. ***p<0.001, compared to pre-treatment. Source data are provided as a Source Data file

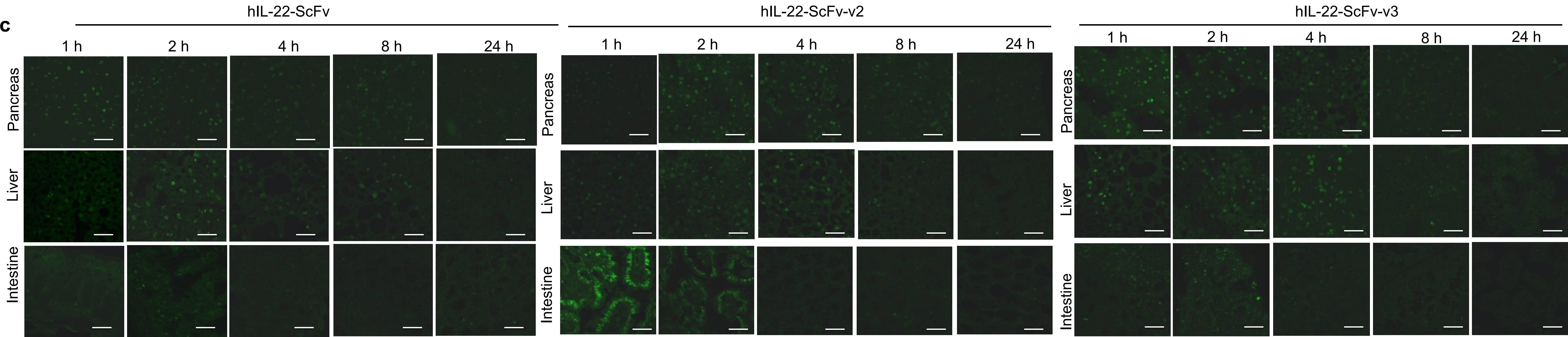
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Variant name	Description	Amino Acid Sequence Underline: Signal Peptide; Grey shading: IL22; Blue shading: scFv; Red text: Fusion Linker; Blue text: Vh/Vk linker	Molecular Weight (kDa)	pI	Extinction Coefficient
hIL-22-ScFv	Human IL22 with (G4S)1 fusion linker and (G4S)3 Vh/Vk linker.	MGWSCIIILFLVATATGVHSAPISSHCRLDKSNFQQPYITNRTFMLAKEASLADNNTDVRLLIGEKLFGHVSMSERCYLMKQVLNFTLEEVLFPQSDRFQPYMQEVVPFLARLSNRLSTCHIEGDDLHIQRNVQKLKDTVKKLGESGEIKAIGELDLLFMSLRNACI GGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSSYAMSWV EQAPGKGLEWVSSITAE GHTHTWYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAKTSYRFDYWGGQGLTVTVSSGGGGSGGGSGGGSGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYKASRLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQKWDPDPRTFGQGTKVEIKR*	42,632	8.89	1.310
hIL-22-ScFv-v2	Expected Monomer Human IL22 with (G4S)3 fusion linker and (G4S)3 Vh/Vk linker.	MGWSCIIILFLVATATGVHSAPISSHCRLDKSNFQQPYITNRTFMLAKEASLADNNTDVRLLIGEKLFGHVSMSERCYLMKQVLNFTLEEVLFPQSDRFQPYMQEVVPFLARLSNRLSTCHIEGDDLHIQRNVQKLKDTVKKLGESGEIKAIGELDLLFMSLRNACI GGGGSGGGSGGGSGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSSYAMSWV WRQAPGKGLEWVSSITAE GHTHTWYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAKTSYRFDYWGGQGLTVTVSSGGGGSGGGSGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYKASRLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQKWDPDPRTFGQGTKVEIKR*	43,263	8.89	1.291
hIL-22-ScFv-v3	Expected Monomer Human IL22 with (G4S)5 fusion linker and (G4S)3 Vh/Vk linker.	MGWSCIIILFLVATATGVHSAPISSHCRLDKSNFQQPYITNRTFMLAKEASLADNNTDVRLLIGEKLFGHVSMSERCYLMKQVLNFTLEEVLFPQSDRFQPYMQEVVPFLARLSNRLSTCHIEGDDLHIQRNVQKLKDTVKKLGESGEIKAIGELDLLFMSLRNACI GGGGSGGGSGGGSGGGSGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSSYAMSWV WRQAPGKGLEWVSSITAE GHTHTWYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAKTSYRFDYWGGQGLTVTVSSGGGGSGGGSGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYKASRLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQKWDPDPRTFGQGTKVEIKR*	43,893	8.89	1.272
	Expected Monomer				

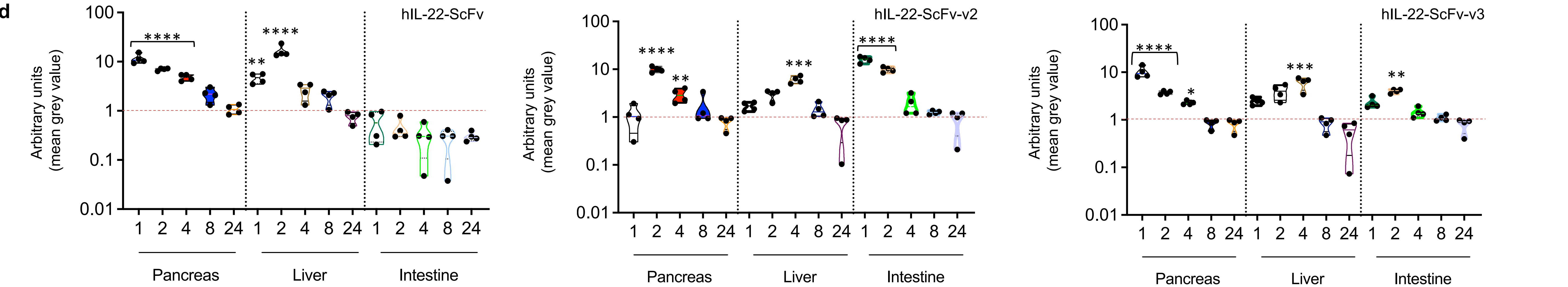
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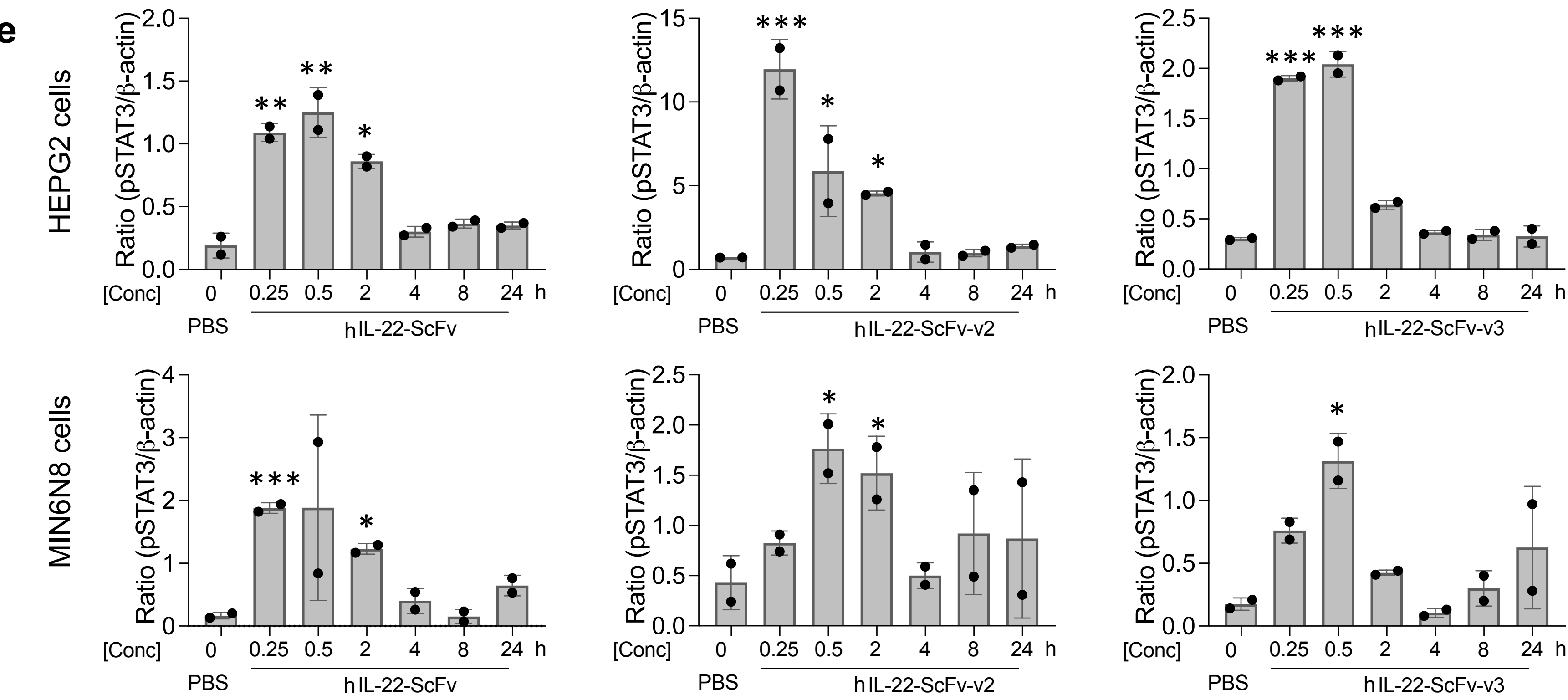
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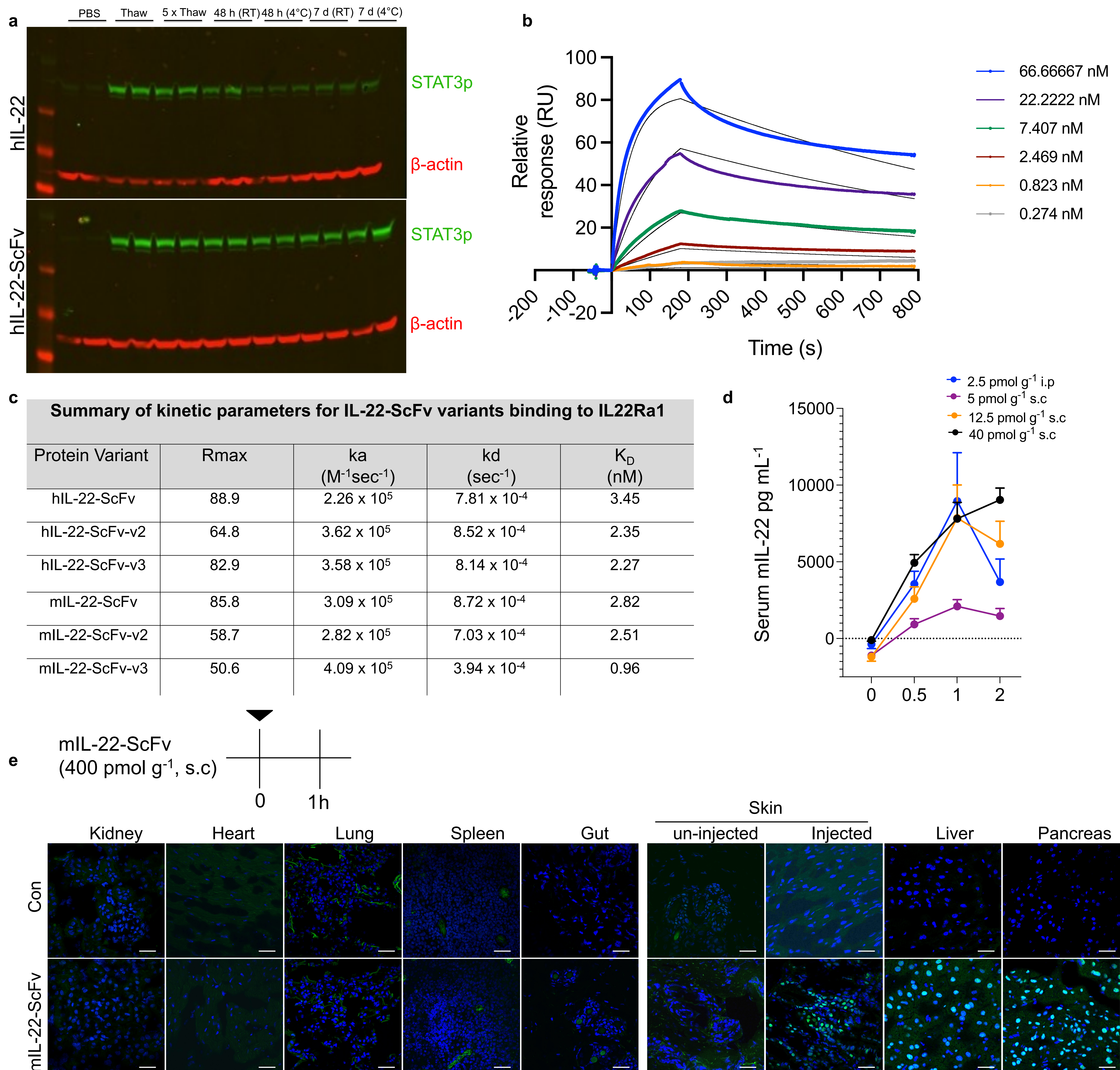
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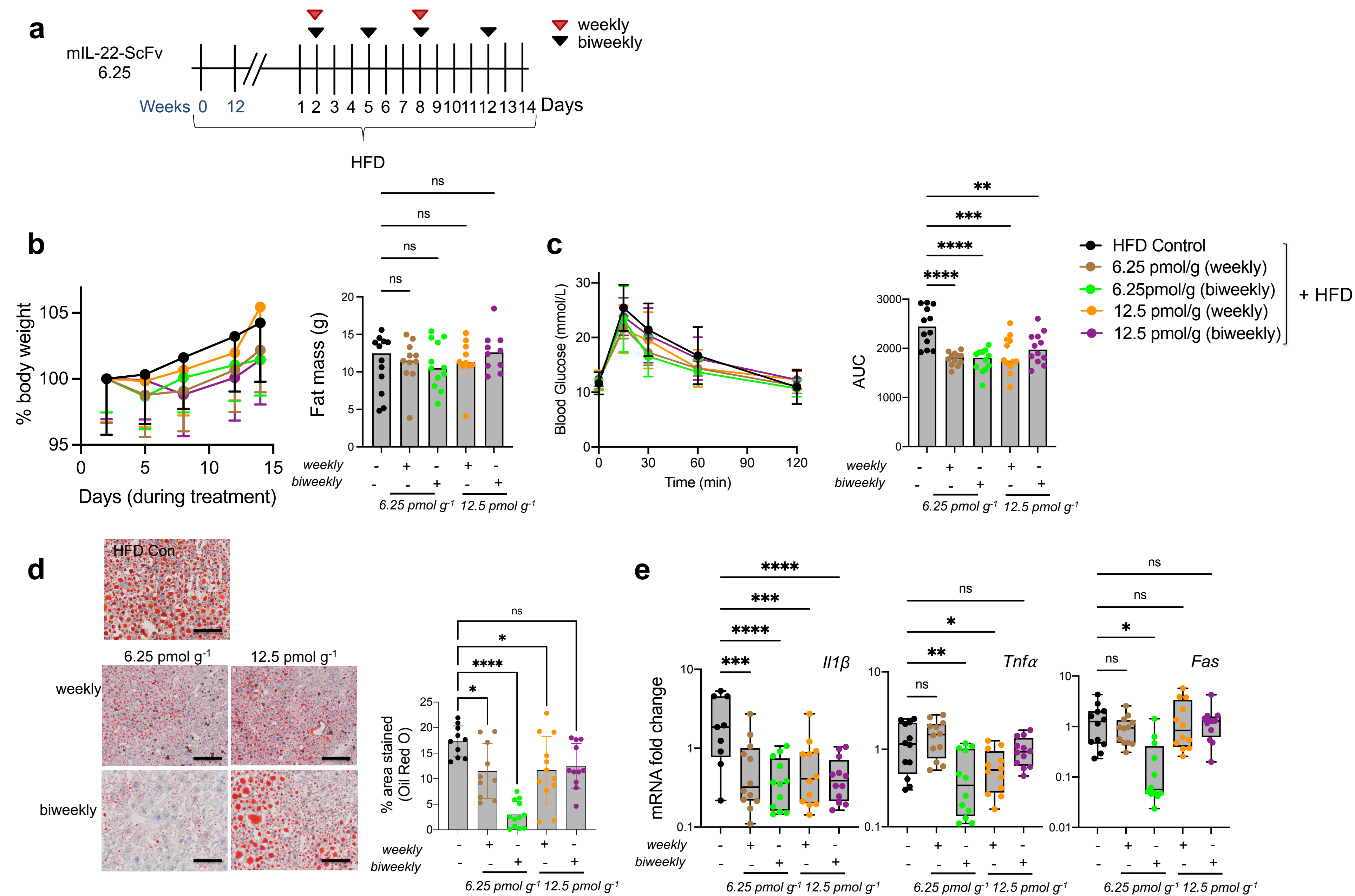
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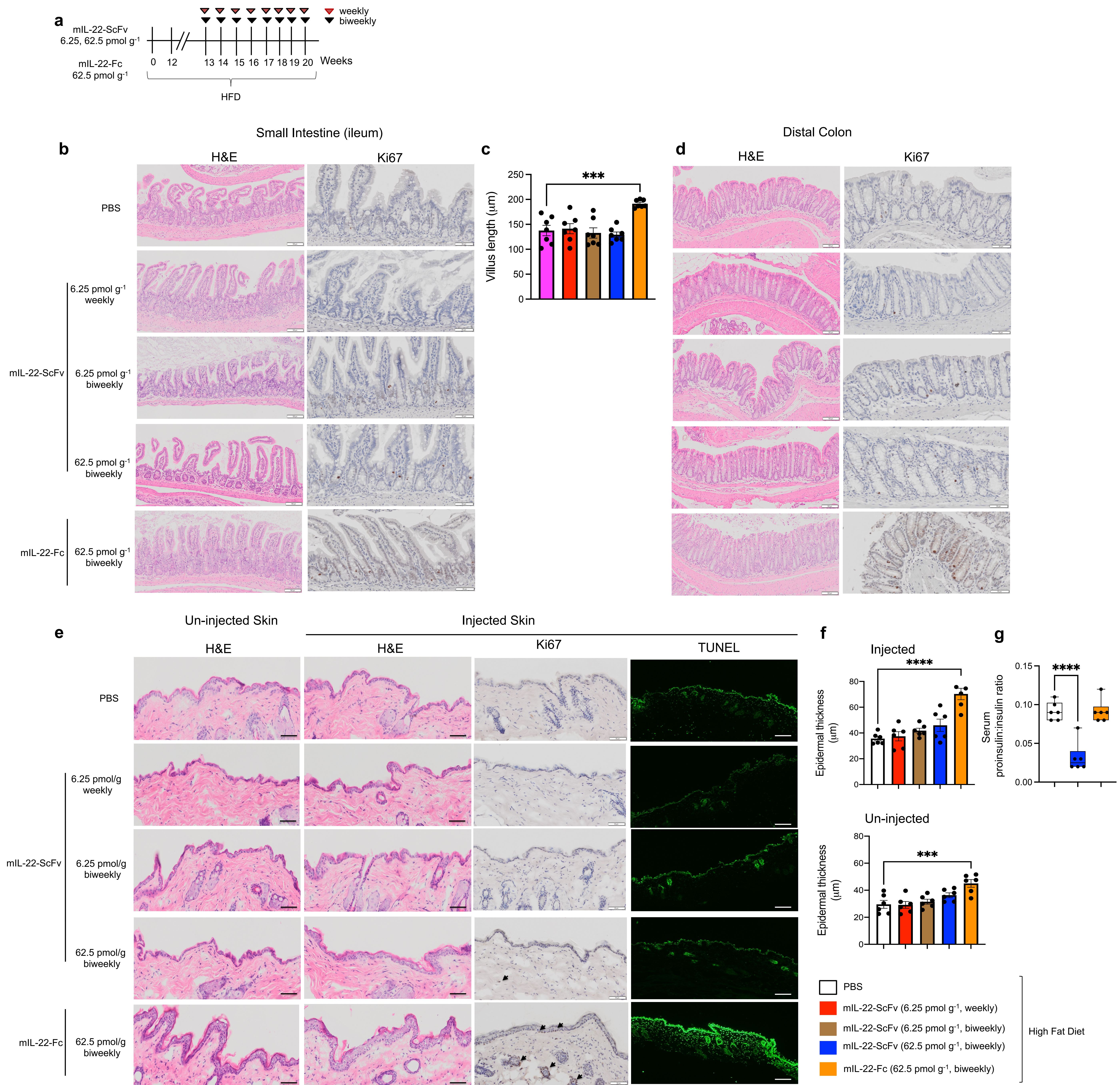
Supplementary Figure 7: Development of IL-22-ScFv variants. IL-22-ScFv was altered to include different linkers between IL-22 and ScFv, sequences shown in (a). WT C57BL/6 animals were kept on a HFD for 12 weeks and subsequently a single 6.25 pmol g⁻¹ dose of hIL-22-ScFv, hIL-22-ScFv-v2 or hIL-22-ScFv-v3 subcutaneously injected. Blood was collected at 1, 2, 4, 8 and 24 h post injection, IL-22 levels were assessed using ELISA, with the IL-22-fusions were used as standards (b). Stat3p was assessed in the pancreas, liver and intestine at 1, 2, 4, 8 and 24 h post hIL-22-ScFv, hIL-22-ScFv-v2 and hIL-22-ScFv-v3 administration (c). Quantification of staining intensity is shown as mean grey value (arbitrary units) as a fold change compared to PBS treated controls (shown as a red-dashed line) (d). n = 4 per group, One-way ANOVA, Bonferroni's post hoc test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to PBS. (e) HEPG2 (hepatoma) and MIN6N8 (insulinoma) were stimulated with IL-22-ScFv variants at 10 pmol mL⁻¹ concentration and STAT3p was assessed at 6 different time points (0.25, 0.5, 1, 4, 8, 24 h) to assess the kinetics of activation compared with PBS treated cells. n = 2 per time point. PBS was used a vehicle control (con) for all experiments. ANOVA, Bonferroni's post hoc test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to control. Scale bars: 50 μm. Source data are provided as a Source Data file



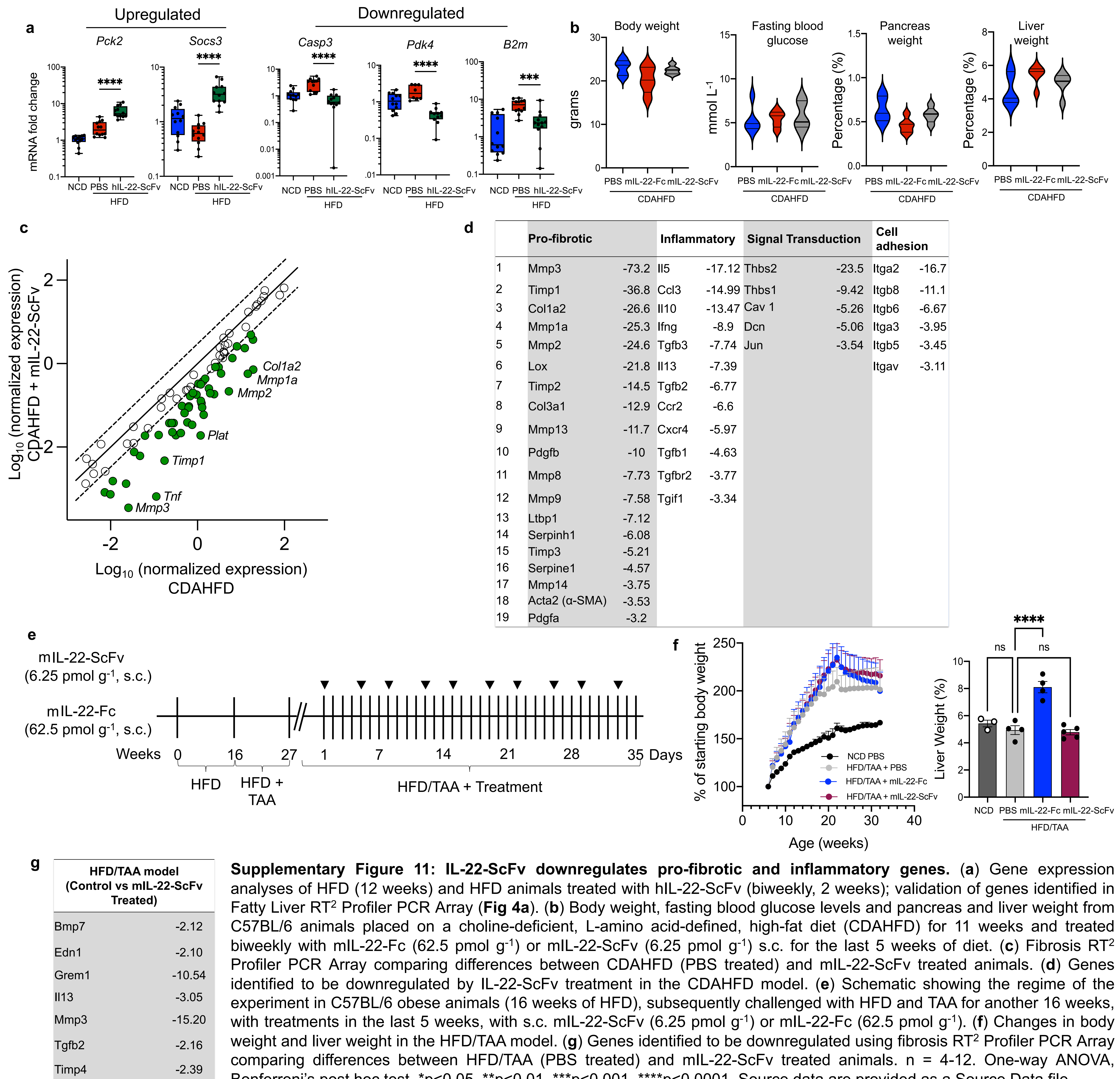
Supplementary Figure 8: IL-22-ScFv is stable, binds to IL-22RA1 and retain targeting at high concentrations. Human IL-22-ScFv and native hIL-22 was diluted at 3-5 pmol mL⁻¹ concentration in PBS and incubated at various time points and temperatures as stated. HEPG2 cells were treated at the same time for 30 mins and western blots to assess STAT3p was conducted (**a**). (**b**) Binding of human IL-22-ScFv to hIL-22RA1 and (**c**) summary kinetics of all human and mouse IL-22-ScFv variants shown in Supplementary Figure 7. (**d**) WT C57BL/6 animals were injected with 5, 12.5 or 40 pmol g⁻¹ of mIL-22-ScFv subcutaneously and this was compared to the therapeutic dose (2.5 pmol g⁻¹) administered i.p. (**e**) C57BL/6 animals were given 400 pmol g⁻¹ of mIL-22-ScFv (s.c), tissue was isolated for Stat3p analyses by immunofluorescence compared with PBS injected controls, confirming targeting. n = 6 per group. Scale bars: 50 μ m. Source data are provided as a Source Data file

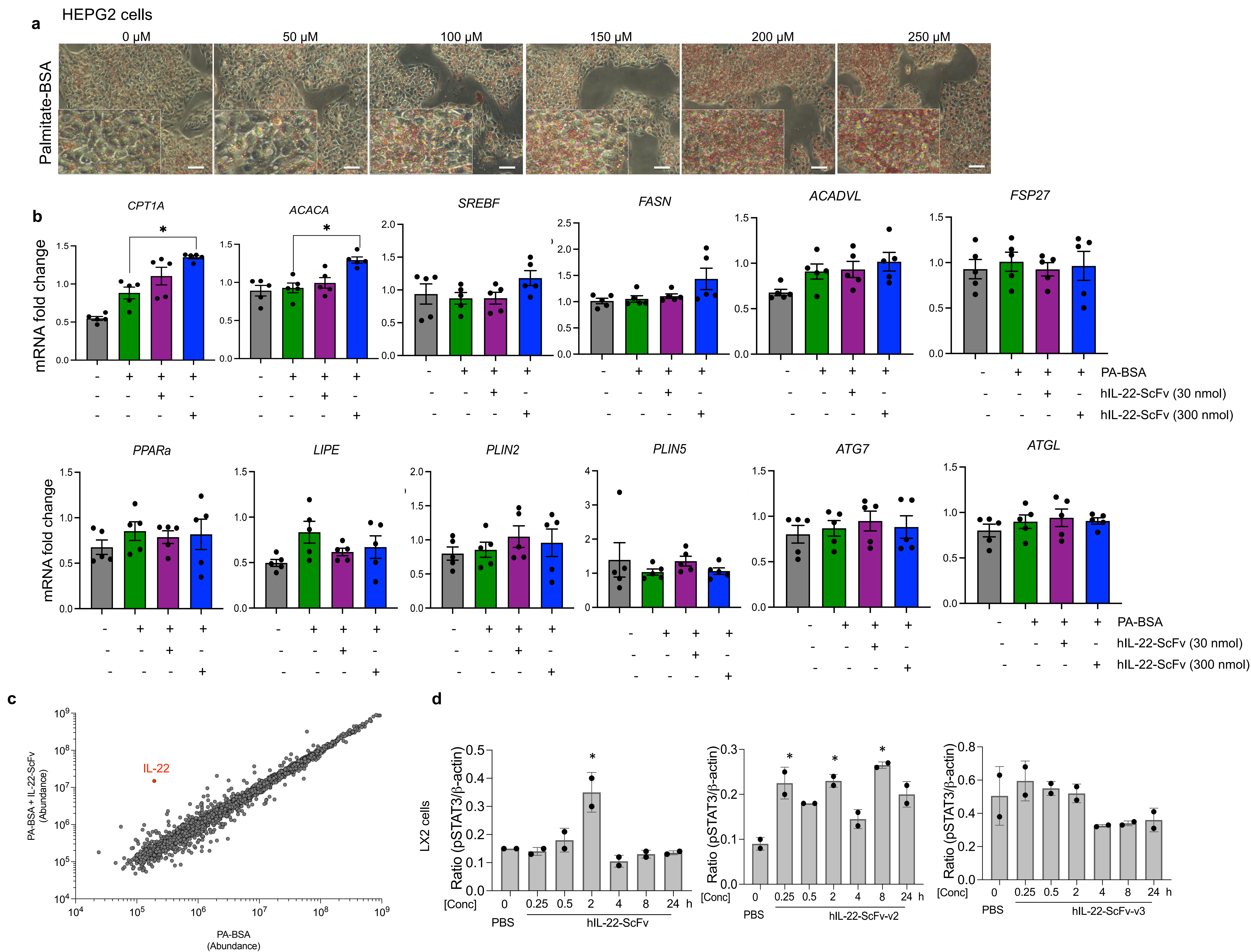


Supplementary Figure 9: mIL-22-ScFv retains targeting and efficacy when administered subcutaneously. WT C57BL/6 animals were kept on a high fat diet (HFD) for 14 weeks and then treated with mIL-22-ScFv at 6.25 or 12.5 pmol g⁻¹, s.c. weekly or biweekly (**a**, schematic) for the last two weeks. (**b**) Body weight (shown as a percentage) and fat mass (measured via NMR) during the treatment, (**c**) Oral glucose tolerance test was assessed, AUC shown after 14 days of treatment. (**d**) Oil Red O staining of liver and percentage of area stained compared with HFD controls; scale bar: 100 μ m. (**e**) Hepatic gene expression analyses of *Il1b*, *Tnfa* and *Fas* in animals after 14 days of treatment. n = 12 per group. One-way ANOVA, Bonferroni's post hoc test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to HFD control. Source data are provided as a Source Data file.



Supplementary Figure 10: WT C57BL/6 animals were kept on a high fat diet (HFD) for 20 weeks and then treated for the last 8 weeks of diet with mIL-22-ScFv at 6.25 or 62.5 pmol g⁻¹, s.c. weekly or biweekly or mIL-22-Fc at 62.5 pmol g⁻¹, biweekly, s.c. (a, schematic). (b) H&E (scale bars: 100 μm) and Ki67 staining (scale bar: 50 μm) and, (c) Villus length measurements in the small intestine. (d) H&E (scale bar: 100 μm) and Ki67 staining (scale bar: 50 μm) in the distal colon. (e) H&E staining (scale bar: 50 μm) of uninjected and injected skin, Ki67 staining (scale bar: 50 μm) showing proliferation and TUNEL staining (scale bar: 100 μm) showing apoptosis. (f) Quantification of epidermis thickness in all treated HFD animals compared to HFD animals treated with PBS. (g) Proinsulin:insulin ratio of HFD animals treated with PBS, or mIL-22-ScFv or mIL-22-Fc at 62.5 pmol g⁻¹, biweekly, s.c. n = 6 per group. One-way ANOVA, Bonferroni's post hoc test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to HFD control. Source data are provided as a Source Data file.





Supplementary Figure 12: hIL-22-ScFv effect on HEPG2, and LX2 cells. (a) HEPG2 cells were treated with increasing concentrations of palmitate and oil red O staining used to determine changes in lipid accumulation; inset x 10 magnification; scale bar: 200 μ m. (b) RT-PCR used to assess the expression of lipolysis genes in HEPG2 cells were treated with Palmitate (200 μ M) to induce lipid accumulation and treated with hIL-22-ScFv (30 or 300 nmol mL⁻¹); n = 4. c, Palmitate stimulated HEPG2 cells were treated with IL-22-ScFv or vehicle only and analysed using mass spectroscopy; which led to identifying 5480 proteins with high confidence. d, LX2 cells treated with 30 nmol mL⁻¹ of hIL-22-ScFv (and variants, described in Supplementary Figure 8) and STAT3p assessed at different times as outlined on the x-axis. One-way ANOVA, Bonferroni's post hoc test. *p<0.05. Source data are provided as a Source Data file

Supplementary Table 1: Demographic information about human pancreatic islet donors.

Donor	Sex	Age (Range)	BMI	History
1	F	60 - 65	35	No substantive history
2	M	60 - 65	29	No substantive history
3	M	55 - 60	26	On medication for Hypertension, Smoker
4	F	60 - 65	27	No substantive history

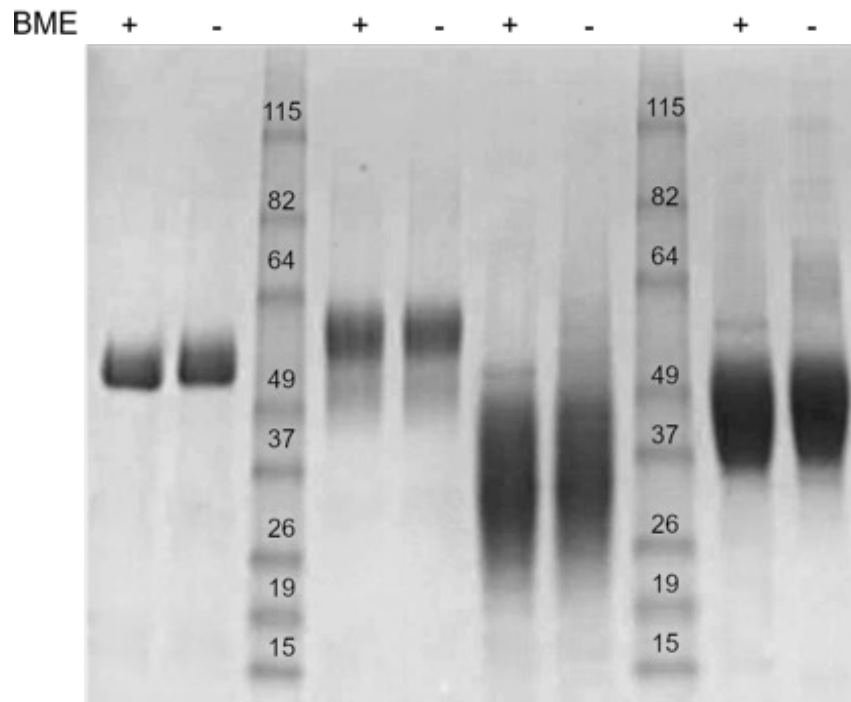
Supplementary Table 2: Human and mouse primer sequences.

Human Primers		
Gene Name	Forward Primer	Reverse Primer
ACACA	TCACACCTGAAGACCTTAAAGCC	AGCCCACACTGCTTGTACTG
ACADVL	ACGATCCCGCCAAGAATGAC	TTGAAACCGATGCTCTGATGG
ATG7	CTGCCAGCTCGCTTAACATTG	CTTGTTGAGGAGTACAGGGTTTT
ATGL	ATGGTGGCATTTCAGACAACC	CGGACAGATGTCACTCTCGC
B-ACTIN	CCTGTACGCCAACACAGTGC	ATACTCCTGCTTGCTGATCC
CPT1A	ATGCGCTACTCCCTGAAAGTG	CGGTGGTTTCTTGGTCGGT
FASN	AAGGACCTGTCTAGGTTTGATGC	TGGCTTCATAGGTGACTTCCA
FSP27	ATTGATGTGGCCCGTGTAACG	CAGCAGTGCAGATCATAGGAAA
LIPE	CCTCAGCCTTCTTCCATGAG	CACAGCTGCATTGCCATAGT
PLIN2	TTGCAGTTGCCAATACCTATGC	CCAGTCACAGTAGTCGTCACA
PLIN5	AAGGCCCTGAAGTGGGTTC	GCATGTGGTCTATCAGCTCCA
PPARα	TTCGCAATCCATCGGCGAG	CCACAGGATAAGTCACCGAGG
SREBF	GCCCCTGTAACGACCACTG	CAGCGAGTCTGCCTTGATG
sXBP-1	GAGTCCGCAGCAGGTGC	CAAAGGATATCAGACTCAGAATCTGAA

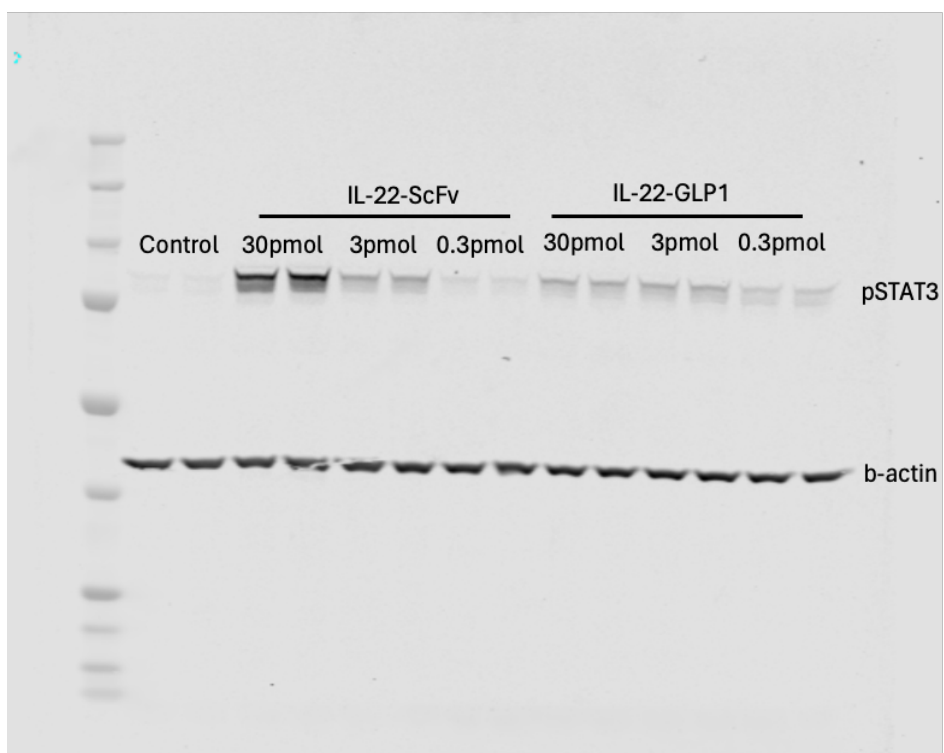
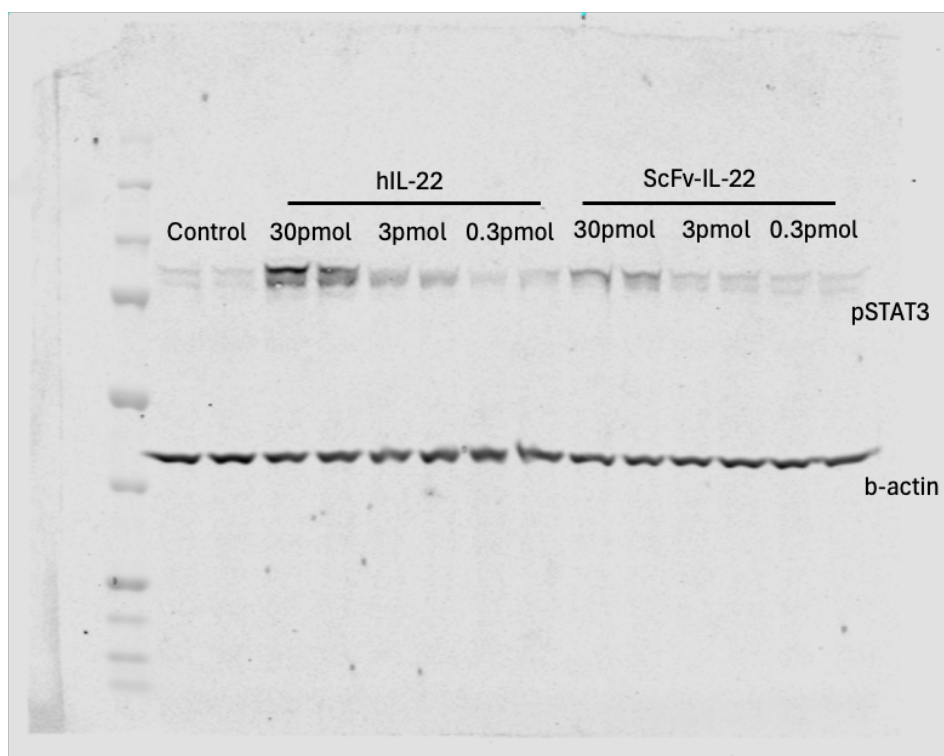
Mouse Primers		
Gene Name	Forward Primer	Reverse Primer
B2m	CCCCACTGAGACTGATACATACG	CGATCCCAGTAGACGGTCTTG
Cd68	TGTCTGATCTTGCTAGGACCG	GAGAGTAACGGCCTTTTTGTGA
Col1a1	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
Col1a2	GTA ACTTCGTGCCTAGCAACA	CCTTTGTCAGAATACTGAGCAGC
Col3a1	CTGTAACATGGAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC
Cpt1a	CTCCGCCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
Elovl6	GAAAAGCAGTTCAACGAGAACG	AGATGCCGACCACCAAAGATA
Fas	GGCATCATTGGGCACTCCTT	GCTGCAAGCACAGCCTCTCT
Grp78	CGCCTCATCGGACGCACTTGG	GCTTGCCGCTGTGCATCATTGA
Hmgcr	AGCTTGCCCGAATTGTATGTG	TCTGTTGTGAACCATGTGACTTC
Il1b	AGAGTTACACTGCCTTTGCCATCC	CCACGTCAATCTTTCCTCTTGCTT
Ldlr	AGTGGCCCCGAATCATTGAC	CTAACTAAACACCAGACAGAGGC
Mmp2	CAAGTTCCCCGGCGATGTC	TTCTGGTCAAGGTCACCTGTC
Nos2	CAGCTGGGCTGTACAAACCTT	CATTGGAAGTGAAGCGTTTCG
Ppia	CAAATGCTGGACCAAACACAAACG	GTTCATGCCTTCTTTCACCTTCCC
Srebp1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
sXbp1	GAGTCCGCAGCAGGTGC	CAAAGGATATCAGACTCAGAATCTGAA
Tata	CCCTCTTCAAAAATTCTCCAC	ACCAACAATCACCAACAGCA
Timp2	TCAGAGCCAAAGCAGTGAGC	GCCGTGTAGATAAACTCGATGTC
Tnfa	CATCTTCTCAA AATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC

Uncropped Western Blot Images

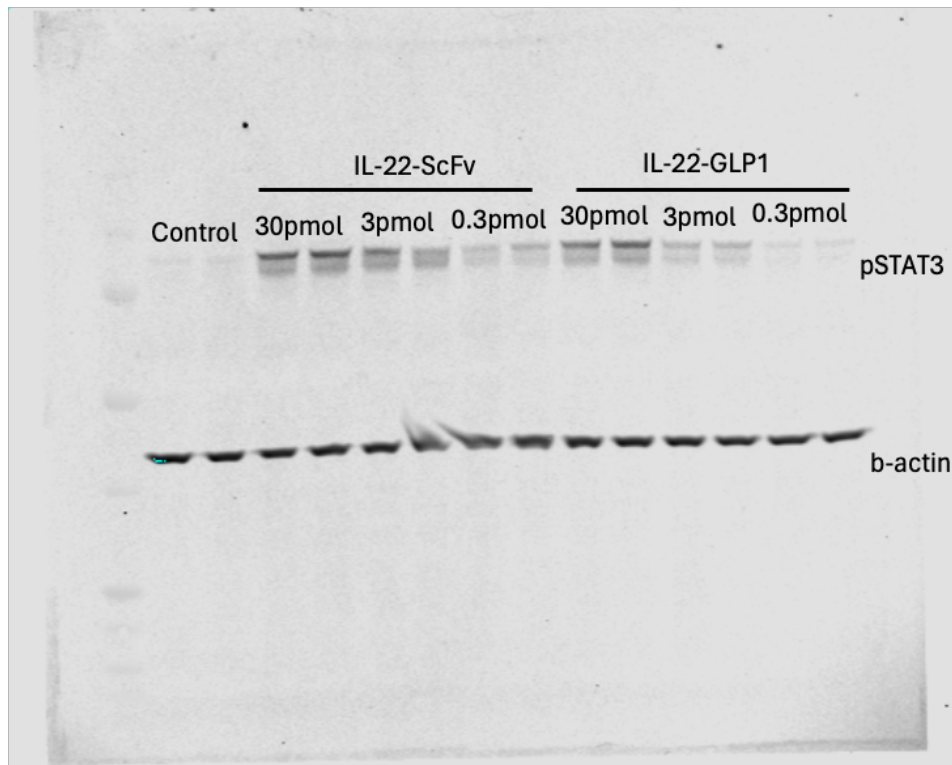
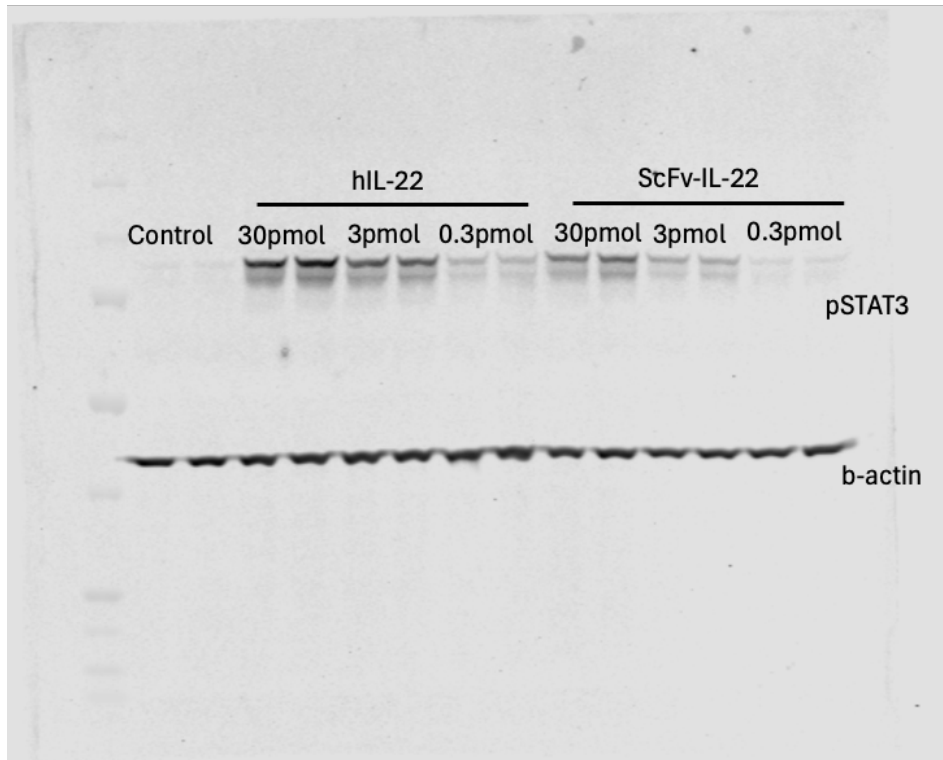
Reducing (+ BME) and non-reducing (- BME) SDS-PAGE of purified IL-22-fusions



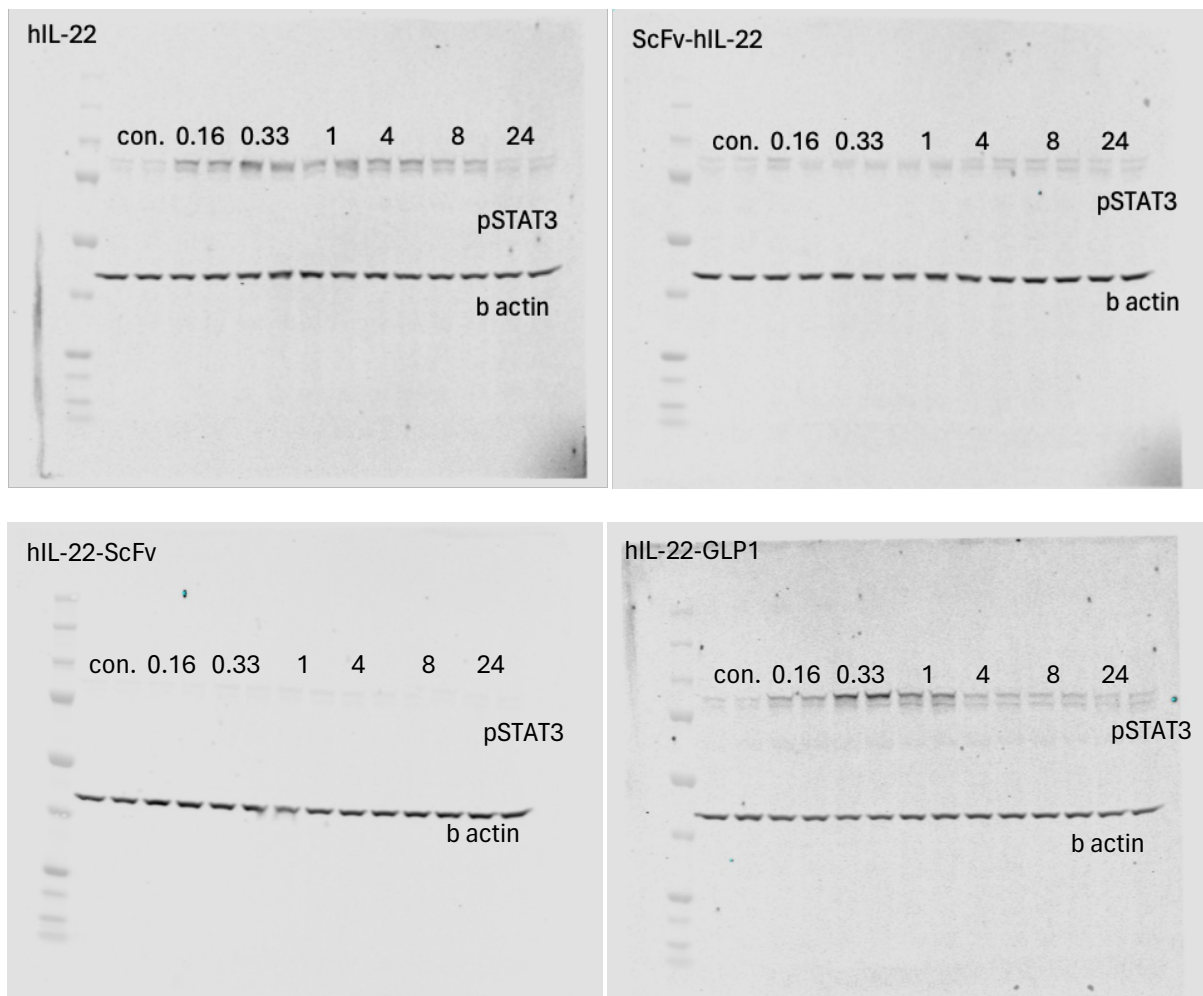
Uncropped western blot – Supplementary Fig. 4b



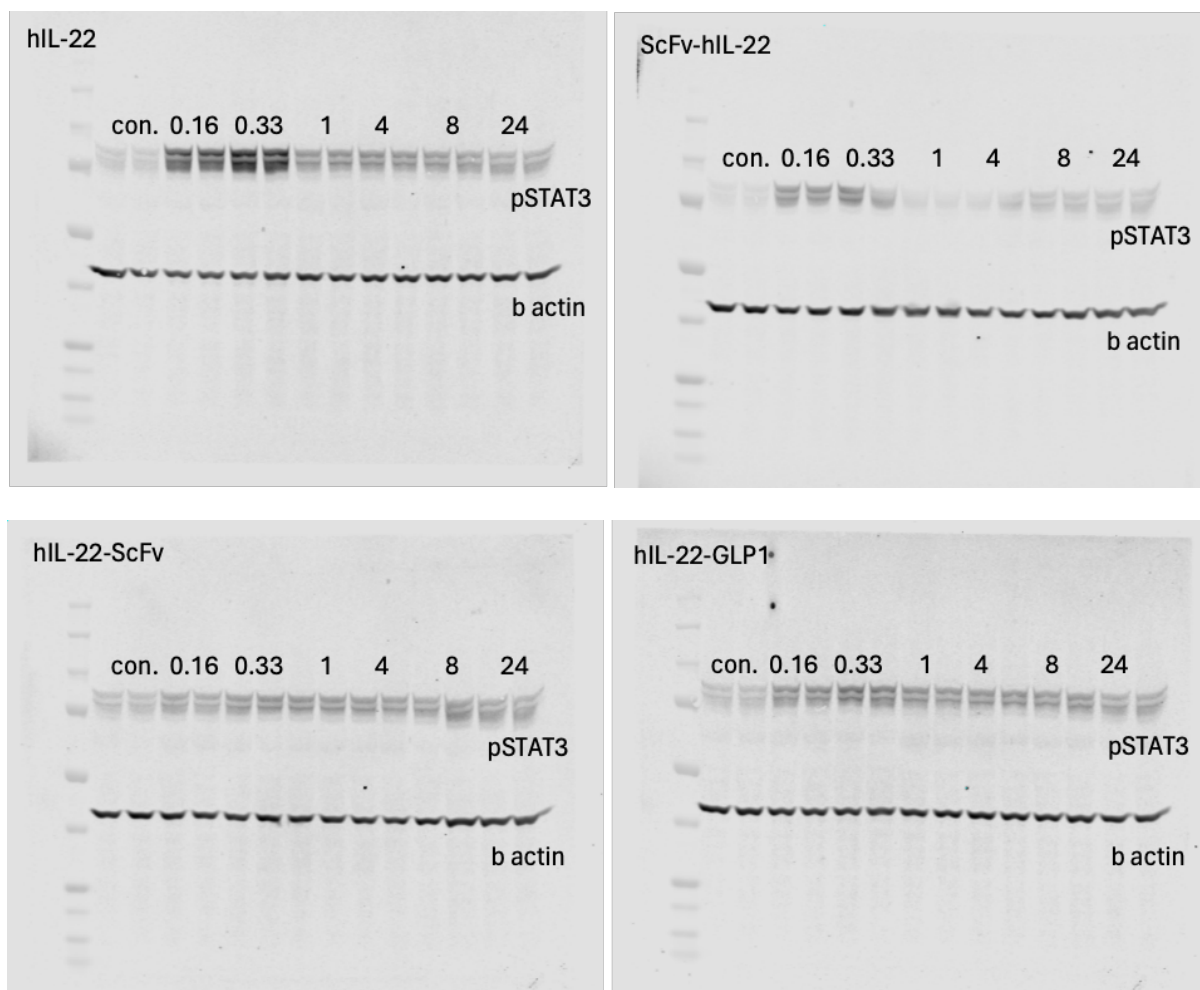
Uncropped western blots – Supplementary Fig. 5a



Uncropped western blots – Supplementary Fig. 5b

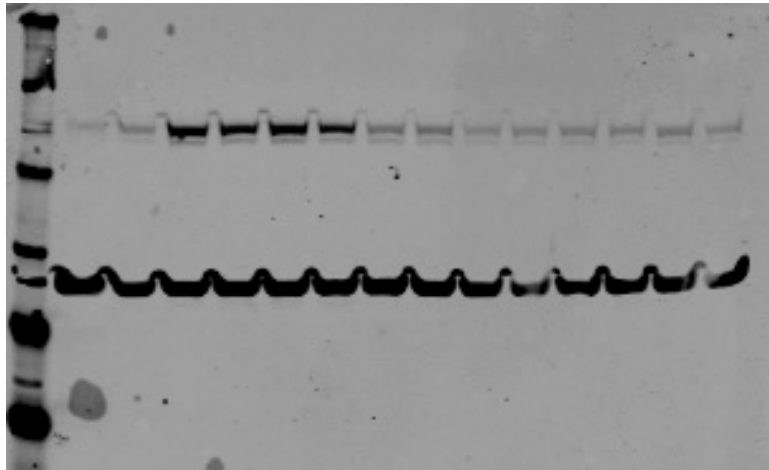


Uncropped western blots – Supplementary Fig. 5c-f



Uncropped western blots – Supplementary Fig. 5g-j

1. IL-22-ScFv



Top-pSTAT3

Bottom-β-actin

Lane 1-2: PBS

Lane 3-4: IL-22-ScFv 15mins

Lane 5-6: IL-22-ScFv -30mins

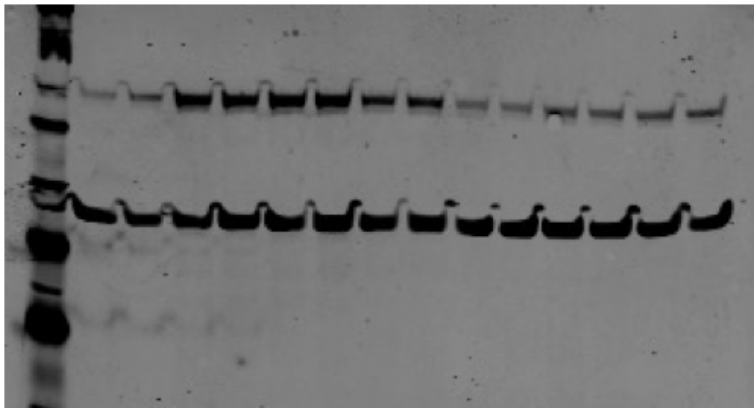
Lane 7-8: IL-22-ScFv 2hrs

Lane 9-10: IL-22-ScFv -4hrs

Lane 11-12: IL-22-ScFv -8hrs

Lane 13-14: IL-22-ScFv -24hrs

2. IL-22-ScFv-v2



• Top-pSTAT3

• Bottom-β-actin

• Lane 1-2: PBS

• Lane 3-4: IL-22-ScFv-v2 15mins

• Lane 5-6: IL-22-ScFv-v2 -30mins

• Lane 7-8: IL-22-ScFv-v2 2hrs

• Lane 9-10: IL-22-ScFv-v2 -4hrs

• Lane 11-12: IL-22-ScFv-v2 -8hrs

• Lane 13-14: IL-22-ScFv-v2 -24hrs

3. IL-22-ScFv-v3



• Top-pSTAT3

• Bottom-β-actin

• Lane 1-2: PBS

• Lane 3-4: IL-22-ScFv-v3 15mins

• Lane 5-6: IL-22-ScFv-v3 -30mins

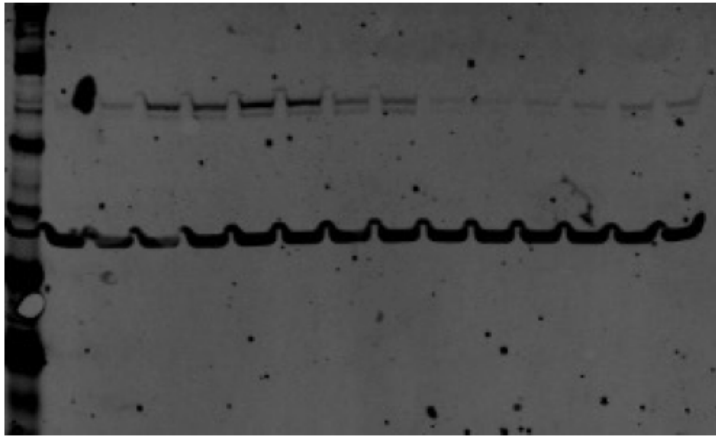
• Lane 7-8: IL-22-ScFv-v3 2hrs

• Lane 9-10: IL-22-ScFv-v3 -4hrs

• Lane 11-12: IL-22-ScFv-v3 -8hrs

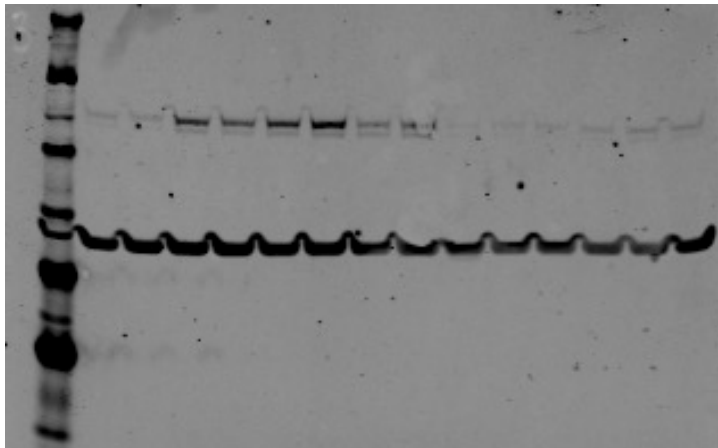
• Lane 13-14: IL-22-ScFv-v3 -24hrs

1. IL-22-ScFv



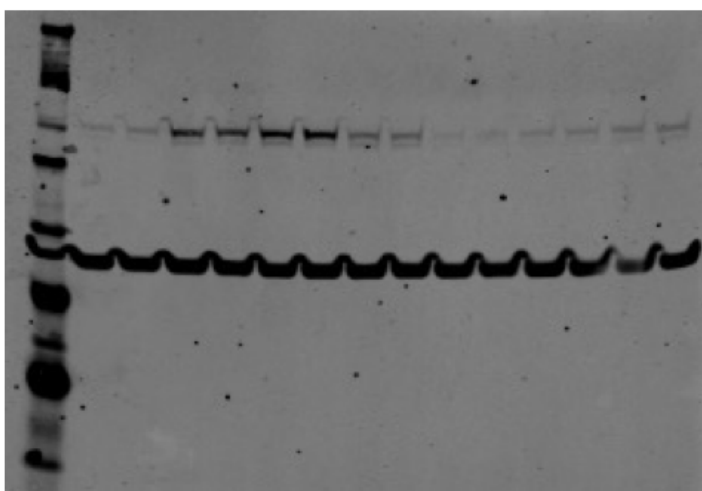
- Top-pSTAT3
- Bottom- β -actin
- Lane 1-2: PBS
- Lane 3-4: IL-22-ScFv-15mins
- Lane 5-6: IL-22-ScFv-30mins
- Lane 7-8: IL-22-ScFv-2hrs
- Lane 9-10: IL-22-ScFv-4hrs
- Lane 11-12: IL-22-ScFv-8hrs
- Lane 13-14: IL-22-ScFv-24hrs

2. IL-22-ScFv-v2



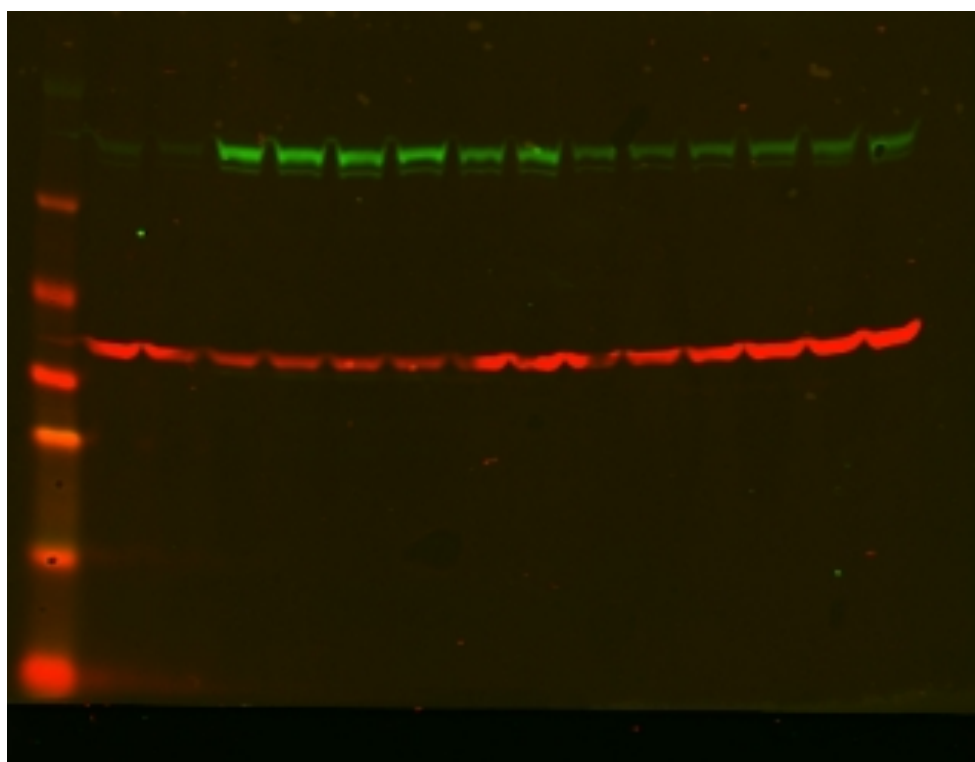
- Top-pSTAT3
- Bottom- β -actin
- Lane 1-2: PBS
- Lane 3-4: IL-22-ScFv-v2-15mins
- Lane 5-6: IL-22-ScFv-v2-30mins
- Lane 7-8: IL-22-ScFv-v2-2hrs
- Lane 9-10: IL-22-ScFv-v2-4hrs
- Lane 11-12: IL-22-ScFv-v2-8hrs
- Lane 13-14: IL-22-ScFv-v2-24hrs

3. IL-22-ScFv-v3



- Top-pSTAT3
- Bottom- β -actin
- Lane 1-2: PBS
- Lane 3-4: IL-22-ScFv-v3-15mins
- Lane 5-6: IL-22-ScFv-v3-30mins
- Lane 7-8: IL-22-ScFv-v3-2hrs
- Lane 9-10: IL-22-ScFv-v3-4hrs
- Lane 11-12: IL-22-ScFv-v3-8hrs
- Lane 13-14: IL-22-ScFv-v3-24hrs

Uncropped western blots – Supplementary Fig. 7e



Green-pSTAT3

Red- β -actin

Lane 1-2: PBS

Lane 3-4: Fresh Thaw

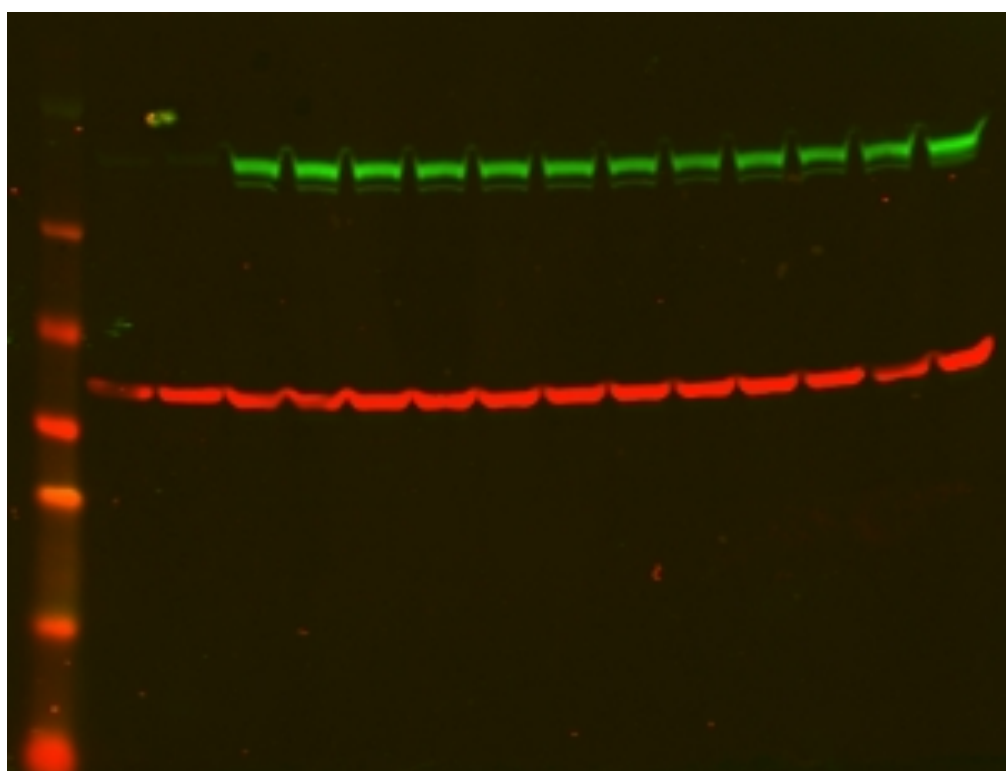
Lane 5-6: Freeze Thaw 5X

Lane 7-8: 48hrs RT

Lane 9-10: 48hrs 4°C

Lane 11-12: 7 days RT

Lane 13-14: 7 days 4°C



Green-pSTAT3

Red- β -actin

Lane 1-2: PBS

Lane 3-4: Fresh

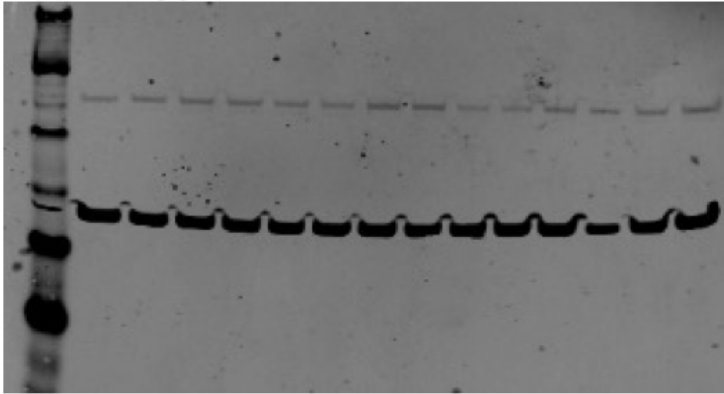
Lane 5-6: Freez

Lane 7-8: 48hrs

Lane 9-10: 48hrs

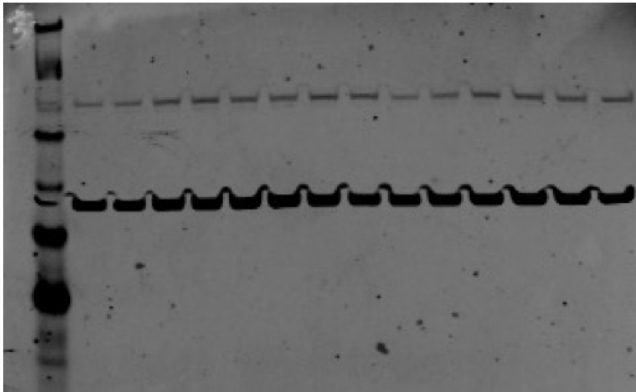
Uncropped western blots – Supplementary Fig. 8a

1. IL-22-ScFv



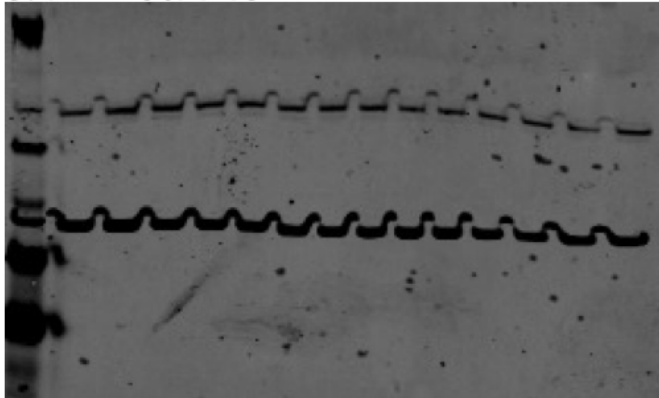
- Top-pSTAT3
- Bottom- β -actin
- Lane 1-2: PBS
- Lane 3-4: IL-22-ScFv-15mins
- Lane 5-6: IL-22-ScFv-30mins
- Lane 7-8: IL-22-ScFv-2hrs
- Lane 9-10: IL-22-ScFv-4hrs
- Lane 11-12: IL-22-ScFv-8hrs
- Lane 13-14: IL-22-ScFv-24hrs

2. IL-22-ScFv-v2



- Top-pSTAT3
- Bottom- β -actin
- Lane 1-2: PBS
- Lane 3-4: IL-22-ScFv-v2 -15mins
- Lane 5-6: IL-22-ScFv-v2 -30mins
- Lane 7-8: IL-22-ScFv-v2 -2hrs
- Lane 9-10: IL-22-ScFv-v2 -4hrs
- Lane 11-12: IL-22-ScFv-v2 -8hrs
- Lane 13-14: IL-22-ScFv-v2 -24hrs

3. IL-22-ScFv-v3



- Top-pSTAT3
- Bottom- β -actin
- Lane 1-2: PBS
- Lane 3-4: IL-22-ScFv-v3 -15mins
- Lane 5-6: IL-22-ScFv-v3 -30mins
- Lane 7-8: IL-22-ScFv-v3 -2hrs
- Lane 9-10: IL-22-ScFv-v3 -4hrs
- Lane 11-12: IL-22-ScFv-v3 -8hrs
- Lane 13-14: IL-22-ScFv-v3 -24hrs

Uncropped western blots – Supplementary Fig. 12d