Supplementary Information Titles

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Article Title: Circulating Angiopoietin-like-4 links proteinuria with hypertriglyceridemia in nephrotic syndrome

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Supplementary Items and Titles

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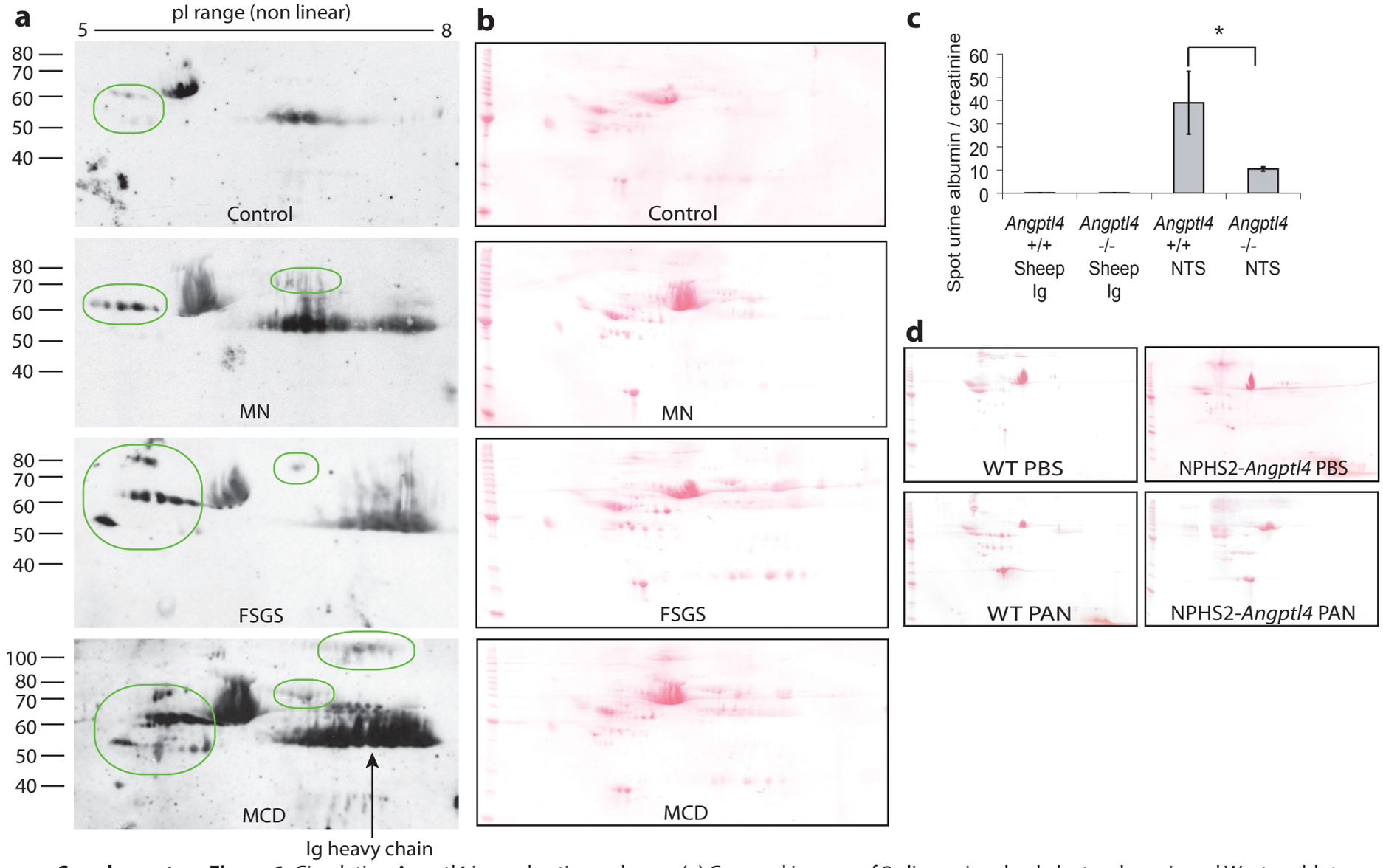
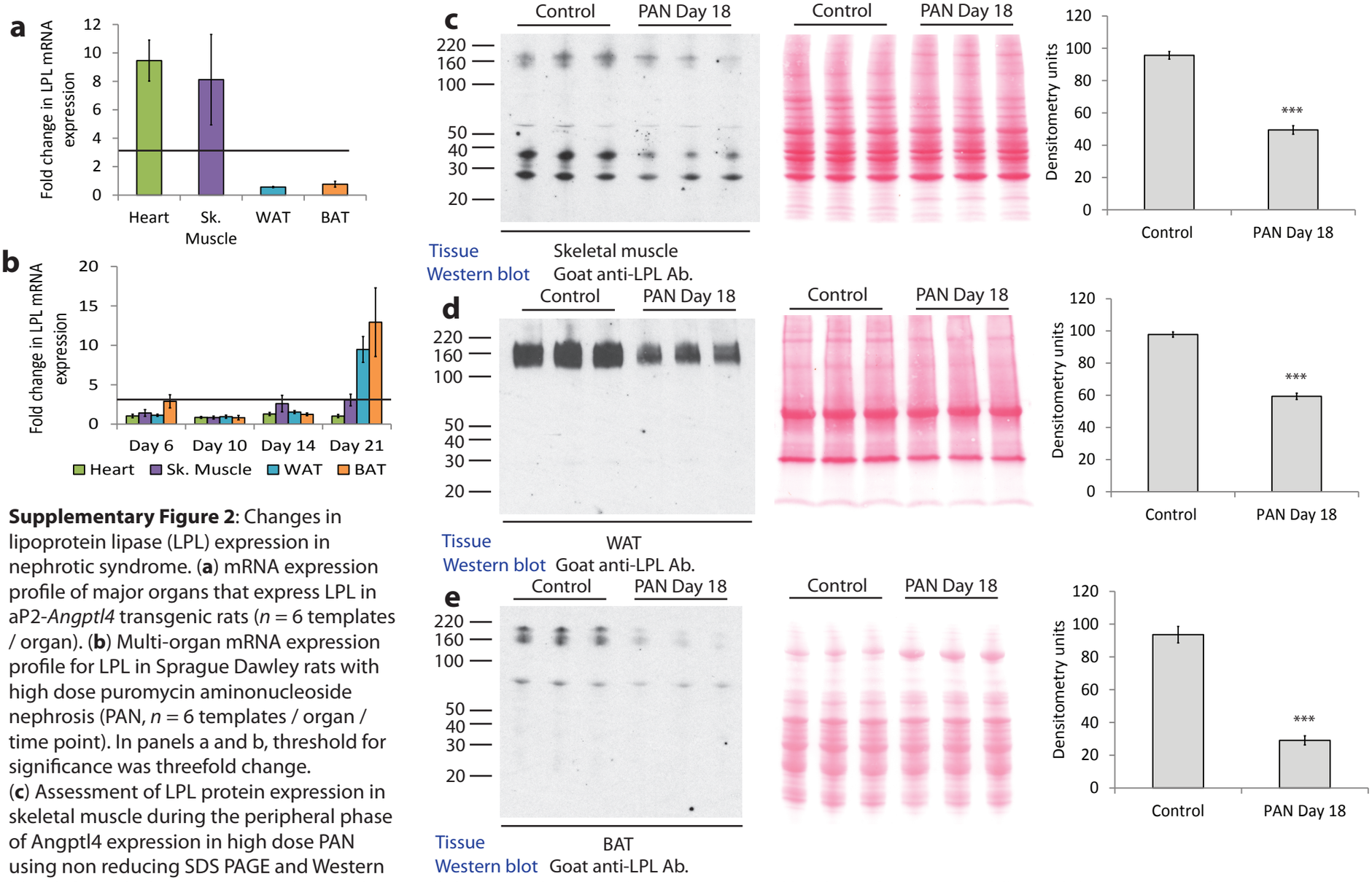
Diagnosis	Age (years)	Sex	Angptl4 (ng per ml)	Diagnosis	Age (years)	Sex	Angptl4 (ng per ml)
Control	42	Male	47.9	FSGS tip lesion	46	Male	378.92
Control	52	Male	50.14	Recurrent FSGS	12	Female	113.46
Control	27	Female	119.28	Recurrent FSGS	46	Female	185.26
Control	31	Female	49.12	Recurrent FSGS	53	Female	166.18
Control	18	Male	44.78	Recurrent FSGS	38	Male	140.08
Control	19	Male	89.34	Recurrent FSGS	44	Female	75.38
Control	31	Female	65.74	Recurrent FSGS	58	Male	495.22
Control	28	Female	71.32				
Control	45	Female	48.32	FSGS#	23	Female	74.22
Control	30	Female	42.88	FSGS#	12	Female	93.94
Control	28	Female	35.46	FSGS#	3	Male	152.68
Control	18	Female	24.78	FSGS#	17	Male	45.48
Control	30	Female	58.76	FSGS#	14	Male	100.34
Control	18	Male	53.06	FSGS#	13	Female	66.7
Control	50	Male	67.7	FSGS#	20	Female	158.24
Control	20	Male	62.14	FSGS#	15	Male	247.44
Control	28	Male	92.54	FSGS#	25	Male	131.02
Control	45	Male	63.26	FSGS#	14	Female	139
Control	39	Male	45.08	FSGS#	14	Male	306.68
				FSGS#	32	Male	45.28
MCD	16	Female	181.16	FSGS#	8	Female	32.04
MCD*	14	Female	316.57	FSGS#	11	Female	95.82
MCD*	14	Female	206.29				
MCD*	4	Male	74.41	Non-HIV CG	25	Female	176.9
MCD*	10	Male	49.81	Non-HIV CG	18	Female	110.24
MCD	19	Male	156.72	Non-HIV CG	29	Male	195.26
MCD	32	Female	144.92	Non-HIV CG	30	Female	228.5
MCD	51	Female	710.26	Non-HIV CG	32	Female	200.02
MCD	52	Male	191.26	Non-HIV CG	34	Female	243.66
MCD	22	Male	571.6	Non-HIV CG	18	Female	591.42
				Non-HIV CG	22	Female	408.48
MN*	59	Male	1195.7	Non-HIV CG	26	Female	330.54
MN*	63	Male	138.62	Non-HIV CG	24	Male	152.68
MN*	31	Female	193.6	Non-HIV CG	16	Female	343.32
MN*	52	Male	92.38	Non-HIV CG	47	Male	473.9
MN	7	Female	128.94	Non-HIV CG	37	Female	168.22
MN	57	Male	86.24	Non-HIV CG	28	Female	74
				Non-HIV CG	18	Male	43.88
FSGS	22	Male	148.78	Non-HIV CG	29	Male	154.12
FSGS	23	Female	158.36	Non-HIV CG	40	Female	137.24
FSGS	28	Male	136.5	Non-HIV CG	55	Male	346.3
FSGS*	49	Male	148.05	Recurrent non-HIV CG	36	Male	64.76
FSGS*	29	Female	141.66				
FSGS*	9	Male	294.17	Transplant non-HIV CG, de novo	35	Male	97.78
FSGS*	8	Male	210.86				

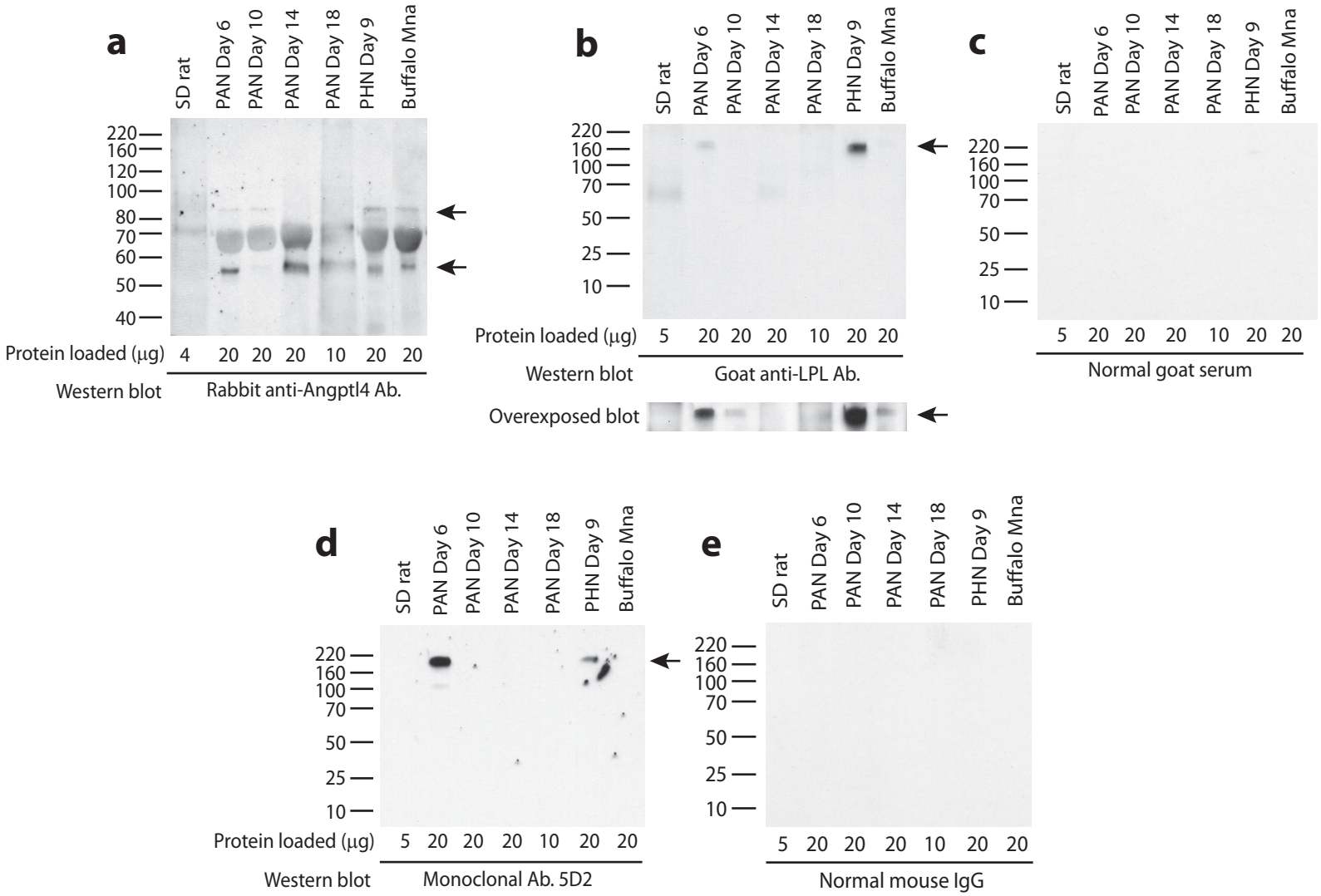
Supplementary table 1: Clinical summary and fasting plasma Angptl4 levels of subjects with nephrotic syndrome and healthy controls subjects. Asterisk indicates plasma obtained from a previously published study[7]. # indicates baseline sample from the FSGS clinical trial[29].



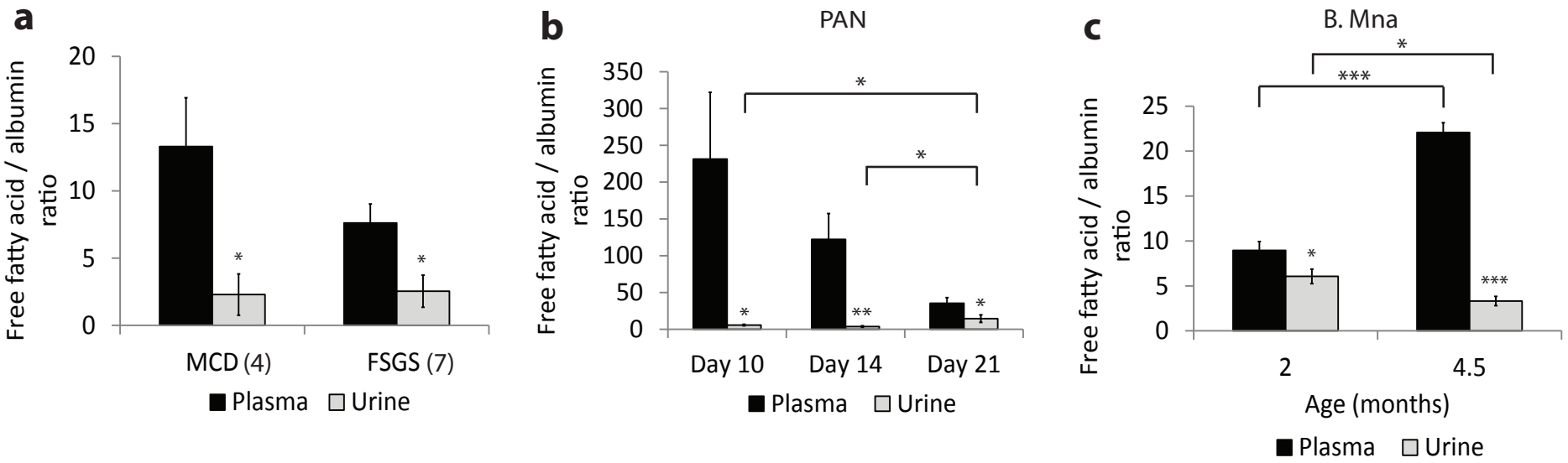
Supplementary Figure 1: Circulating Angptl4 in nephrotic syndrome. (**a**) Cropped images of 2-dimensional gel electrophoresis and Western blots of plasma from representative individuals with membranous nephropathy (MN), focal and segmental glomerulosclerosis (FSGS) and minimal change disease (MCD) using an anti-Angptl4 antibody. Angptl4 spots are enclosed in green circles / ovals. (**b**) Images of ponceau red stained nitrocellulose membranes corresponding to Western blots shown in panel a. (**c**) Albuminuria in *Angptl4*$^{+/+}$ and *Angptl4*$^{-/-}$ mice 48 hours after injection of γ2-nephrotoxic serum (NTS), corresponds to **Fig. 1h** ($n = 4$ mice / group). Image is reproduced from the on line supplement of Reference 7. Similar result was seen on Day 2 of study shown in **Fig. 5i**. (**d**) Images of ponceau red stained nitrocellulose membranes used for Western blot in **Fig. 2d**. Error bars are s.e.m. t test, 2 way, * $P < 0.05$.



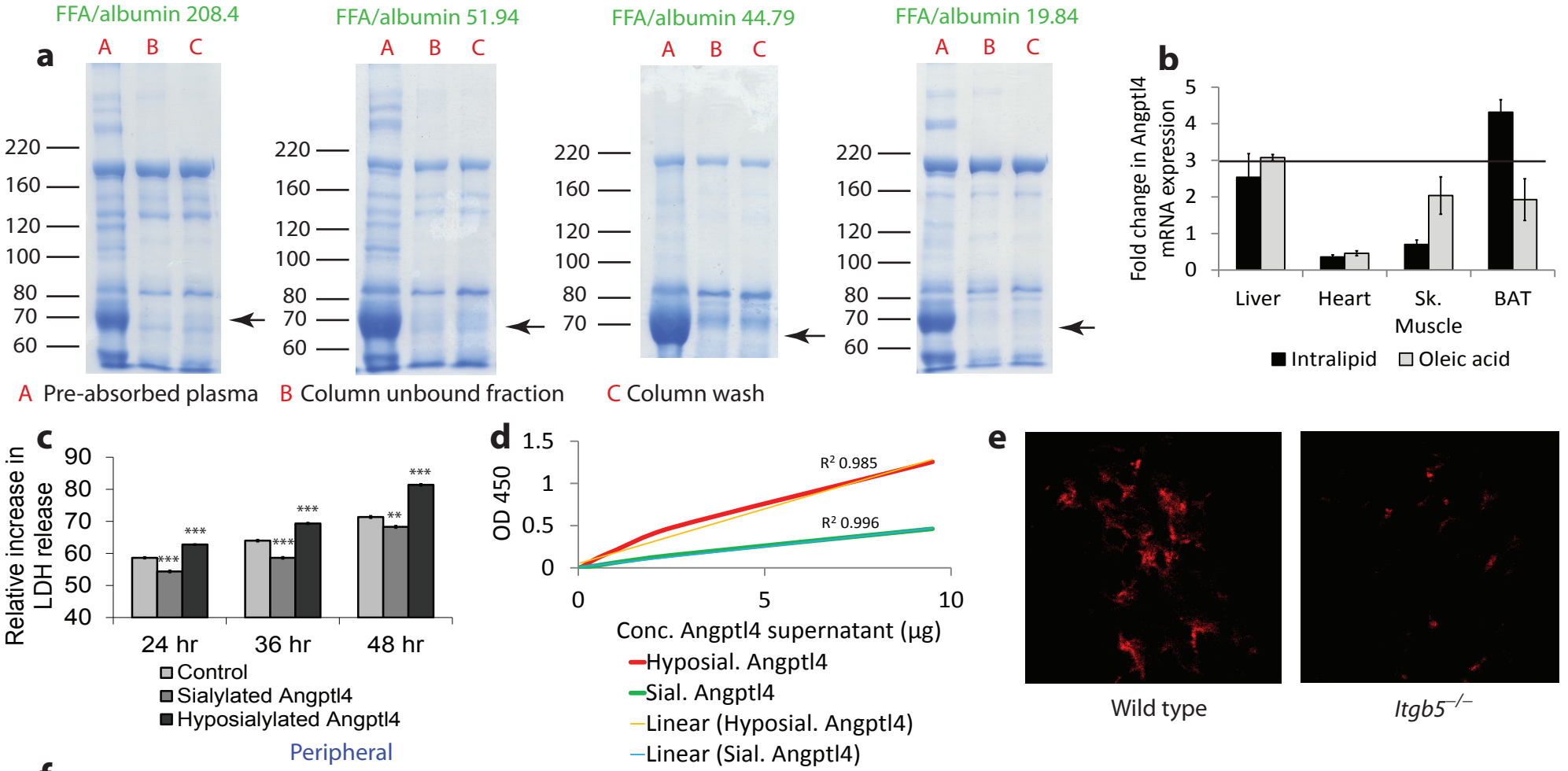
Supplementary Figure 2: Changes in lipoprotein lipase (LPL) expression in nephrotic syndrome. (**a**) mRNA expression profile of major organs that express LPL in aP2-*Angptl4* transgenic rats ($n = 6$ templates / organ). (**b**) Multi-organ mRNA expression profile for LPL in Sprague Dawley rats with high dose puromycin aminonucleoside nephrosis (PAN, $n = 6$ templates / organ / time point). In panels a and b, threshold for significance was threefold change. (**c**) Assessment of LPL protein expression in skeletal muscle during the peripheral phase of Angptl4 expression in high dose PAN using non reducing SDS PAGE and Western blot (left), including ponceau red stained nitrocellulose membrane to demonstrate equal loading of protein (middle) and densitometry of LPL monomers and oligomers (right, sum of all bands). (**d**) Assessment of LPL protein expression in white adipose tissue (WAT) during the peripheral phase of Angptl4 expression in PAN using non reducing SDS PAGE and Western blot (left), including ponceau red stained nitrocellulose membrane to demonstrate equal loading of protein (middle) and densitometry of LPL monomers and oligomers (right). (**e**) Assessment of LPL protein expression in brown adipose tissue (BAT) during the peripheral phase of Angptl4 expression in PAN using non reducing SDS PAGE and Western blot (left), including ponceau red stained nitrocellulose membrane to demonstrate equal loading of protein (middle) and densitometry of LPL monomers and oligomers (right, sum of all bands). Error bars are s.e.m. t test, 2 way, *** $P < 0.001$.



Supplementary Figure 3: Urinary loss of Angptl4 and lipoprotein lipase (LPL) in nephrotic syndrome. (**a**) Representative reducing SDS PAGE and Western blots of urine from normal Sprague Dawley (SD), puromycin aminonucleoside nephrosis (PAN), passive Heymann nephritis (PHN), and Buffalo Mna rats. Black arrows point towards Angptl4 bands. Albumin blush is also noted in PAN, PHN and Buffalo Mna rats between 65 and 70 kDa. (**b**) Non-reducing SDS PAGE and Western blot of urine from nephrotic rats using goat anti LPL antibody to assess for urinary loss of LPL (arrow). The overexposed blot cropped at the level of LPL is shown at the bottom. (**c**) Image of Western blot shown in panel b after being stripped and incubated with normal goat serum. (**d**) Non-reducing SDS PAGE and Western blot of nephrotic rat urine using anti-LPL monoclonal antibody 5D2 to identify active LPL (arrow). (**e**) Image of Western blot shown in panel d after being stripped and incubated with normal mouse IgG.



Supplementary Figure 4: Differences between plasma and urinary free fatty acids (FFA) in human and experimental nephrotic syndrome. Albumin is a carrier protein for FFA. (**a**) Urine and plasma FFA (measured in μM) to albumin (measured in gm L^{-1}) ratio in a subset of individuals with minimal change disease (MCD) and focal and segmental glomerulosclerosis (FSGS) in which both samples were obtained during the same clinic visit. The number of subjects is shown in brackets. (**b**) Plasma and urine FFA/albumin ratio in puromycin aminonucleoside nephrosis (PAN) during the peripheral phase on Angptl4 expression (n = 4 values / group). (**c**) Plasma and urine FFA/albumin ratio before (age 2 months, *n* = 4 values / group) and after (age 4.5 months, *n* = 19 values / group) the development of nephrotic range proteinuria in Buffalo Mna (B. Mna) rats. Error bars are s.e.m. t test, 2 way, except panel c age 2 months is 1 way, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.



Supplementary Figure 5: Mechanisms of Angptl4 upregulation and action. (**a**) GelCode blue stained reducing SDS PAGE of human plasma from **Fig. 3e**, before and after affinity absorption of albumin to assess for albumin depletion (arrow) in the column unbound and first column wash fractions. (**b**) Changes in peripheral organ Angptl4 mRNA expression ($n = 6$ templates / organ / group) in 10 month old Buffalo Mna rats after 3 days of treatment with Intralipid or oleic acid relative to control saline treated animals. Threshold for significance was threefold change (horizontal line). (**c**) Comparison of the effects of recombinant sialylated rat Angptl4 shown in **Fig. 5c** with recombinant hyposialylated Angptl4 on apoptosis induced in cultured rat glomerular endothelial cells using H_2O_2 induced oxidative stress ($n = 3$ readings / group). Statistical comparisons were made with corresponding control groups. (**d**) In vitro plate adhesion assays to study the interaction of sialylated and hyposialylated Angptl4 with purified $\alpha_v\beta_5$ ($n = 3$ readings / value). Linear regression slopes are superimposed. (**e**) Confocal images of glomeruli using anti-V5 antibody (red) to detect presence of recombinant rat Angptl4-V5 two hours after injection into wild type (left) and *Itgb5*$^{-/-}$ (right) mice. (**f**) Plasma Angptl4 levels during the peripheral phase of Angptl4 production in γ2-nephrotoxic serum (NTS) injected wild type and *Itgb5*$^{-/-}$ mice shown in **Fig. 5d,e** ($n = 5$ readings / group). Statistical comparisons were made with Day 0 values. Error bars are s.e.m. t test, 2 way, * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$.

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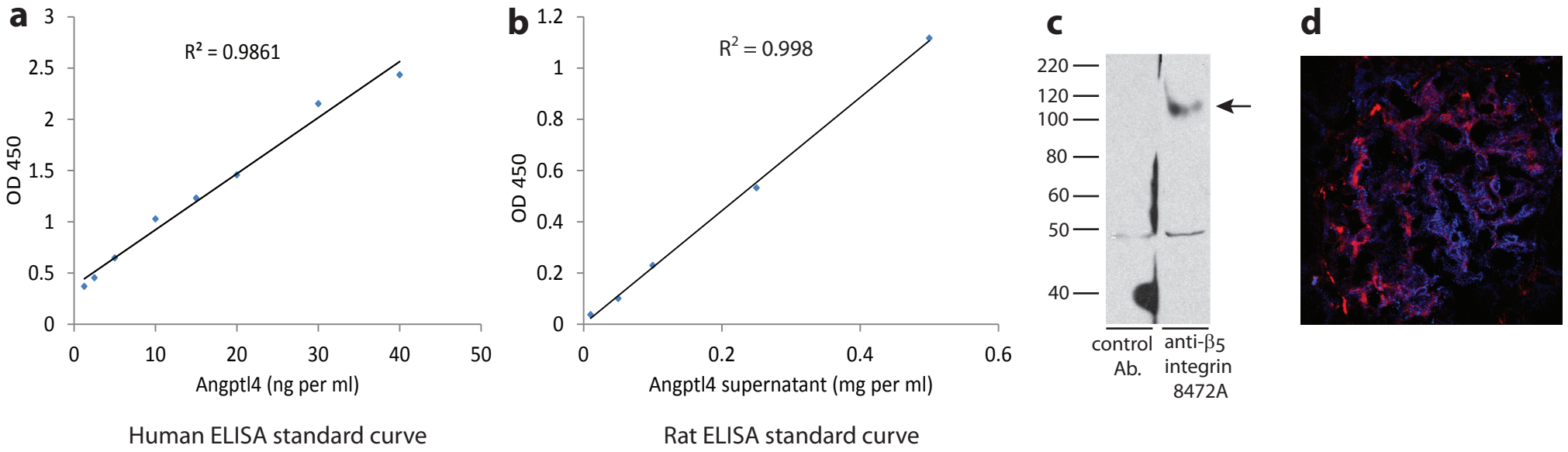

Supplementary Figure 6: Methods section data. (**a**) Standard curve for human Angptl4 ELISA. (**b**) Standard curve for rat Angptl4 ELISA. (**c**) Characterization of the anti-β_5 integrin antibody 8472A by reducing SDS PAGE and Western blot of rat glomerular protein. (**d**) Confocal imaging to demonstrate co-localization (magenta color) of the anti-β_5 integrin antibody (red, detected using donkey anti-rabbit IgG) with glomerular endothelium (blue, labeled with mouse anti-PECAM1 antibody) 3 hours after intravenous injection into a Sprague Dawley rat.

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