

RTN1A-MEDIATED ENDOPLASMIC RETICULUM STRESS IN PODOCYTE INJURY AND DIABETIC NEPHROPATHY

Ying Fan^{1,+}, Jing Zhang^{1,+}, Wenzhen Xiao², Kyung Lee², Zhengzhe Li², Jiejun Wen¹, Li He¹, Dingkun Gui¹, Rui Xue¹, Guihua Jian¹, Xiaohua Sheng¹, John Cijiang He^{2, 3,*} and Niansong Wang^{1,*}

¹Department of Nephrology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China; ²Department of Medicine, Division of Nephrology, Icahn School of Medicine at Mount Sinai, NY United States; and ³Renal Section, James J Peter Veterans Administration Medical Center, Bronx, NY, United States

⁺Ying Fan and Jing Zhang contributed equally to the study.

***Corresponding author:**

1. Niansong Wang, M.D.

Department of Nephrology

Shanghai Jiao Tong University Affiliated Sixth People's Hospital

600 Yishan Road, Shanghai, 200233, China

Tel: +86-021-64369181, Fax: +86-021-64701361

Email: wangniansong2012@163.com

2. John Cijiang He, M.D.

Division of Nephrology, Box 1243

Mount Sinai School of Medicine

One Gustave L Levy Place

New York, NY 10029

Tel: 212-659-1703, Fax: 212-987-0389

Email: cijiang.he@mssm.edu

Supplementary Table S1:

	db/m-sham-16wk	db/db-sham-16wk	db/db-sham-20wk	db/db-Unx-16wk
Body Weight (BW,g)	26.27±1.69	54.53±4.70*	55.00±6.04*	46.43±4.02*
Kidney Weight (KW,mg)	156.8±5.75	239.0±20.78*	222.6±13.63*	369.0±10.86*#◊
KW/BW ratio (mg/g)	5.99±0.50	4.43±0.81	4.11±0.72*	7.98±0.56*#◊
Blood glucose (mM)	8.0±1.2	27.6±1.9*	25.7±1.2*	27.3±4.2*
sCr (mg/dl)	0.25±0.09	0.43±0.11	0.62±0.13*	0.98±0.10*#◊
UACR (ug/mg)	54.8±12.6	1074.1±171.5*	1473.9±181.9*	2011.1±130.3*#◊

Supplementary Table S1. Characteristics of db/m and db/db mice. A graphic presentation of urinary albumin-to-creatinine ratio in db/m and db/db mice. Data are expressed as mean ± SEM. *P<0.05 compared with db/m-sham-16wk mice; *P<0.05 vs db/m-sham-16wk groups; #P<0.05 vs db/db-sham-16wk mice; ♦P<0.05 vs db/db-sham-20wk mice (n=8 for each group).

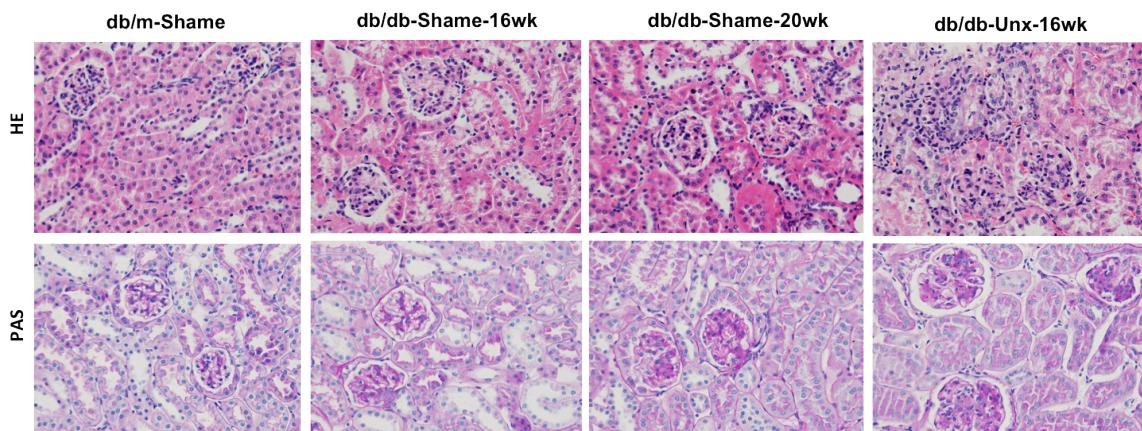
Supplementary Table S2: Primer sequences

Species	Primers	Sequence
Human	H-RTN1A-QF1	TTGCCATGGAAACTGCATCC
	H-RTN1A-QR1	TGTAGAACCTCCTGAGGTG
	H-GRP78-QF1	AACCGCTGAGGCTTATTGG
	H-GRP78-QR1	TAGGCCAGCAATAGTCCAG
	H-CHOP-QF1	AAGGCACTGAGCGTATCATG
	H-CHOP-QR1	TGCTTCAGGTGTGGTGATG
Mouse	M-RTN1A-QF1	ATGGAAACTGCATCCACA
	M-RTN1A-QR1	AAAAGTATGCAGAGTCCTC
	M-GRP78-QF1	AGGCTAACAGAGAGCCTTGTCT
	M-GRP78-QR1	TCCAACACTTCTGGACAGG
	M-CHOP-QF1	TTCACCTGGAGACGGTG
	M-CHOP-QR1	CGCAGGGTCAAGAGTAGTG
	M-bim-QF1	CCTACAGACAGAACCGCAAGC
	M-bim-QR1	CATTGAGGGTGGTCTTCAGC
	M-bax-QF1	GGTTGCCCTCTTCTACTTTGC
	M-bax-QR1	GCCGCTCACGGAGGAAG
	M-bcl2-QF1	CTACCGTCGTGACTTCGCAG
	M-bcl2-QR1	CCCACCGAACTCAAAGAAGG
	M-WT1-QF1	ACAACGCCCATCCTCTGC
	M-WT1-QR1	TACAAGAGTCGGGGCTACTCC
	M-nephrin-QF1	CAAGAAGTCGCTCATCCTGAAC
	M-nephrin-QR1	CCAAACACACCAGCCTCACC
	M-Synaptopodin-QF1	TGACATCCAGCCAACACC
	M-Synaptopodin-QR1	CTGGCCGTCTGTTGACC

Supplementary Table S3: List of antibodies

Antibodies	Company	Cat No	Species	Notes
RTN1A	ABCAM	ab8957	Mouse	Monoclonal
GRP78	Cell Signaling	#3177	Rabbit	Monoclonal
CHOP	Cell Signaling	#2895	Mouse	Monoclonal
Cleaved caspase-3	Cell Signaling	#9664	Rabbit	Monoclonal
PERK	Cell Signaling	#3192	Rabbit	Monoclonal
P-PERK	SANTA CRUZ	sc-32577	Rabbit	Polyclonal
Synaptopodin	ABCAM	ab101883	Rabbit	Polyclonal
P57	SANTA CRUZ	sc-8298	Rabbit	Polyclonal

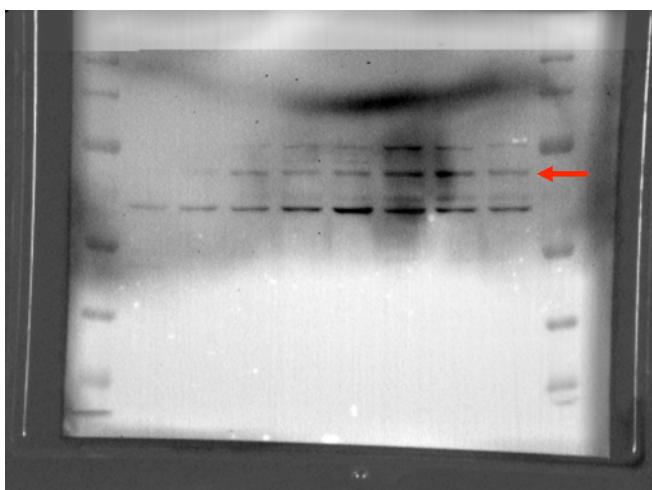
Supplementary Figure S1



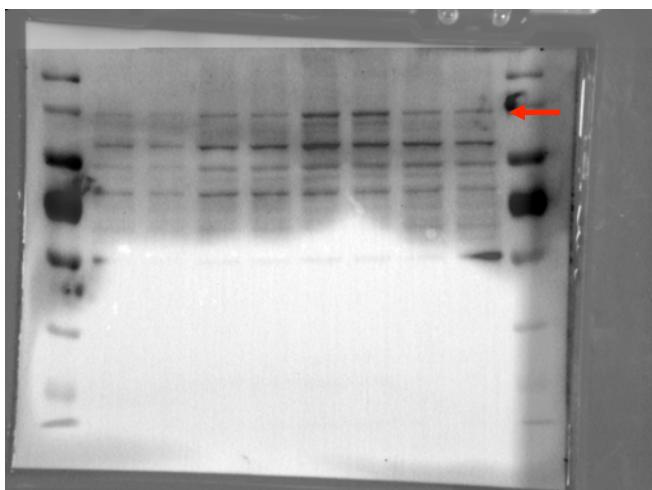
Supplementary Fig. S1: Unilateral nephrectomy accelerated kidney injury in db/db mice. db/db mice and db/m mice underwent unilateral nephrectomy (Unx) or sham operation (sham) at age of 8 weeks. Representative images of haematoxyline-eosin (HE) & periodic acid–Schiff (PAS) stained kidney sections of db/m and db/db mice at 16 weeks of age (original magnification, $\times 400$).

Supplementary Figure S2: Full-length gels and blots in the main article

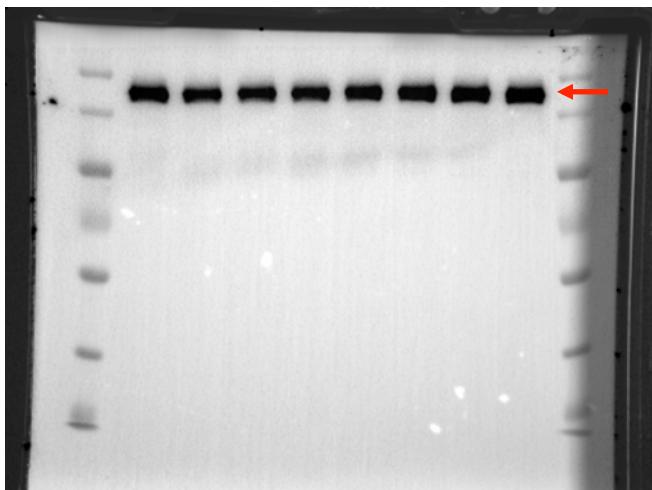
Fig. 2A



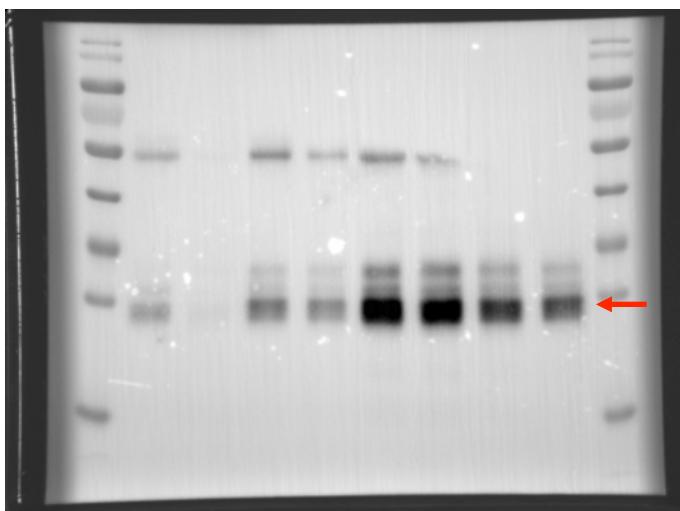
GRP78, MW 78KD



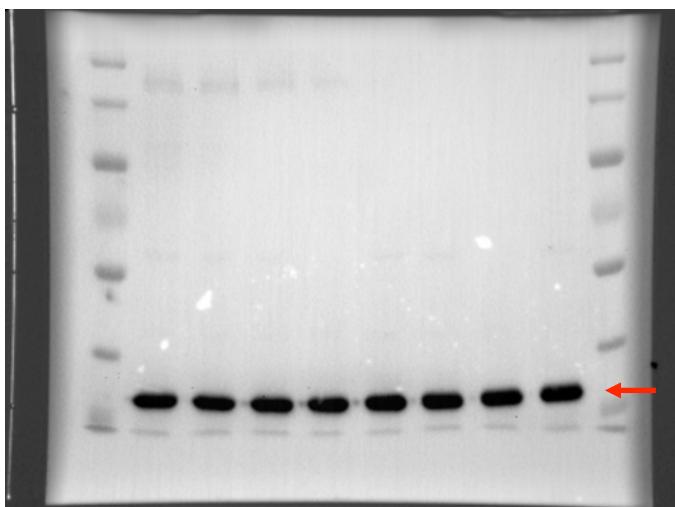
p-PERK, MW 125KD



T-PERK, MW 140KD



CHOP, MW 27KD



GAPDH, MW 36KD

Fig. 4A

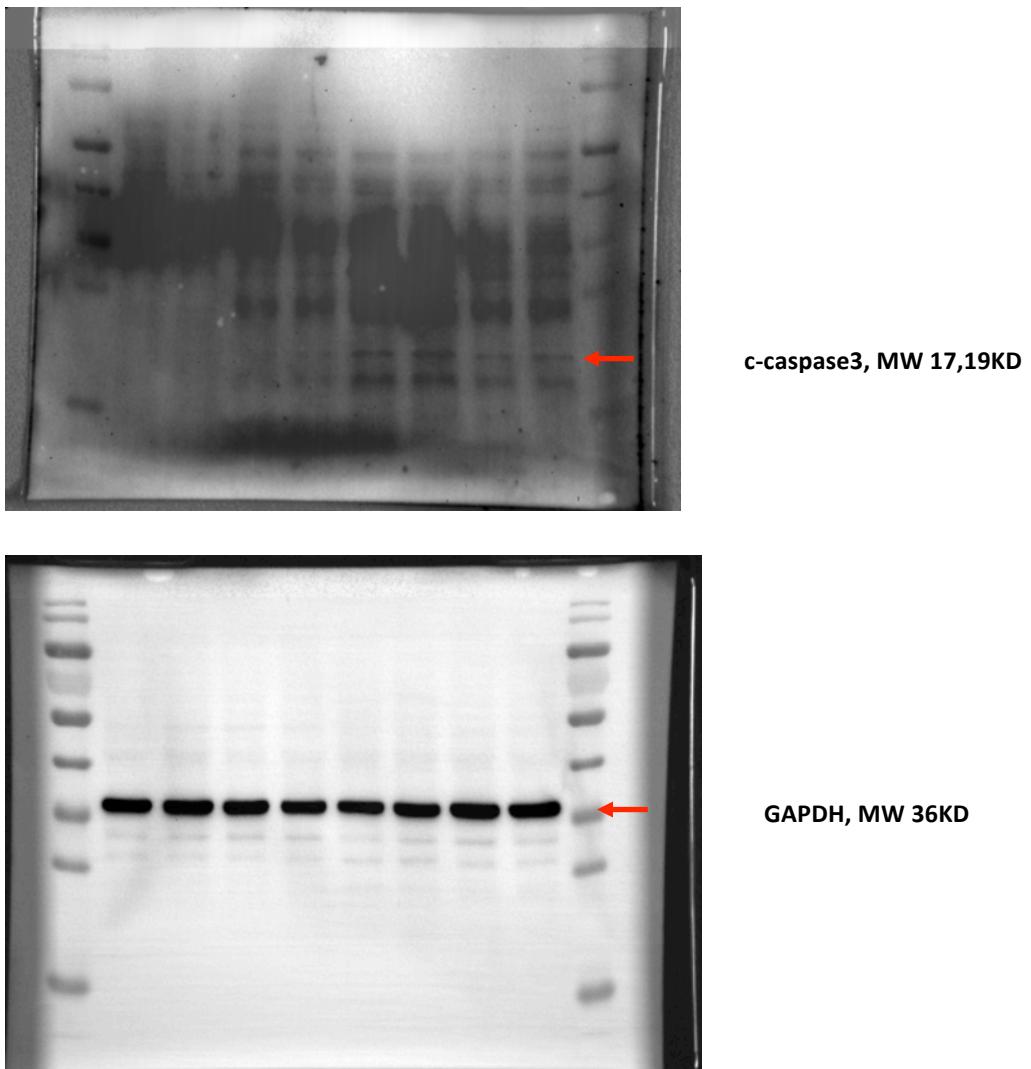
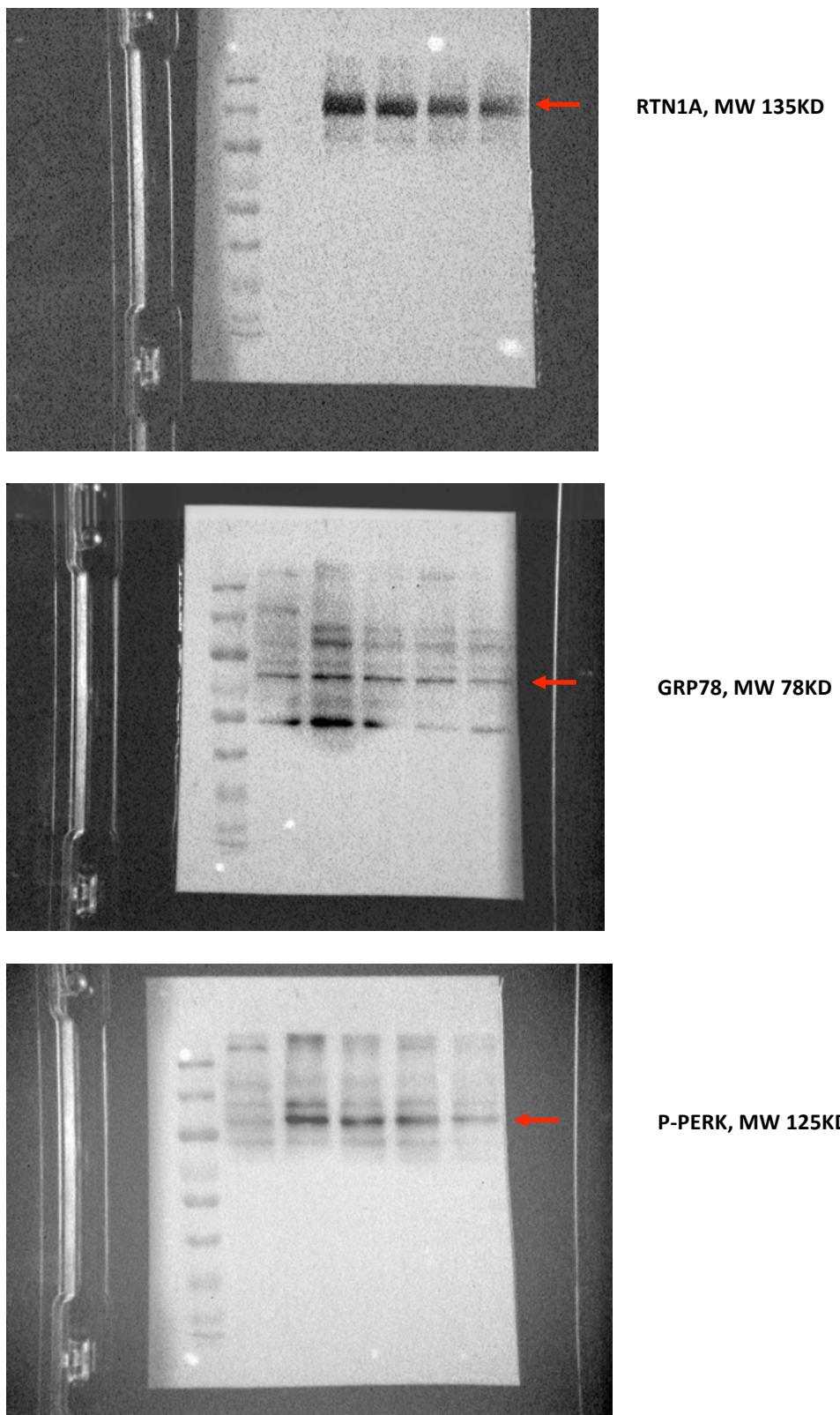
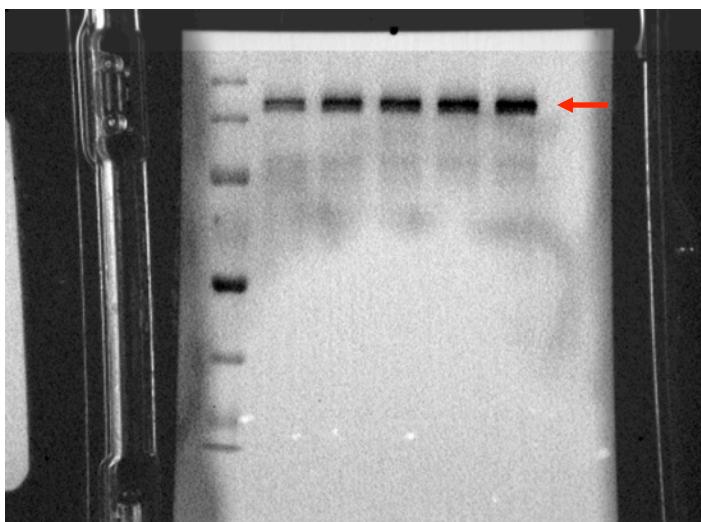
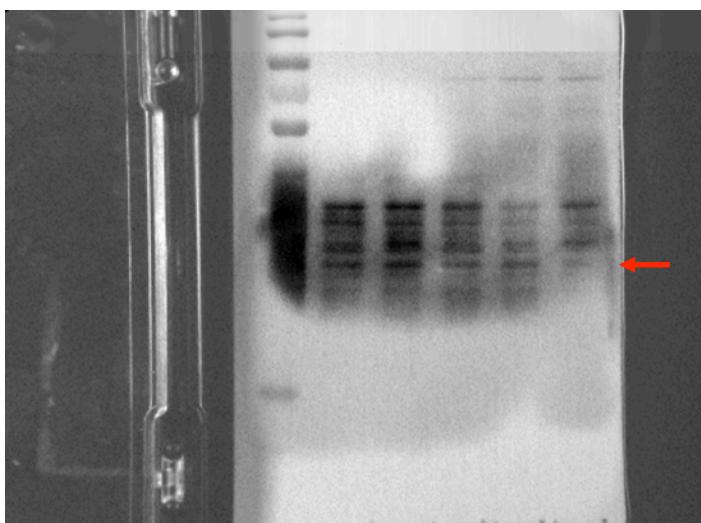


Fig. 6A

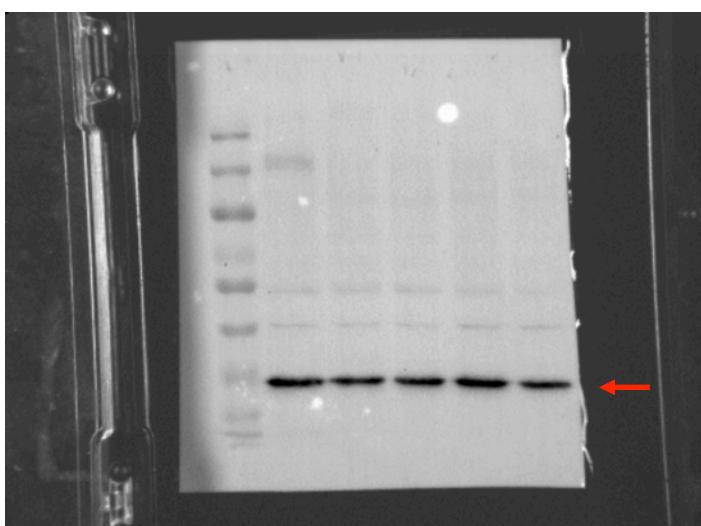




T-PERK, MW 140KD



CHOP, MW 27KD



GAPDH, MW 36KD

Fig. 6D

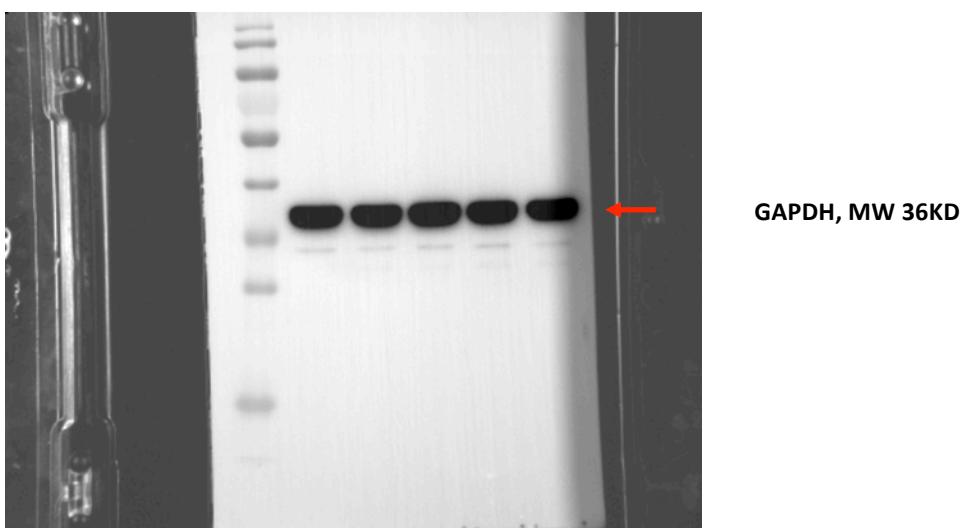
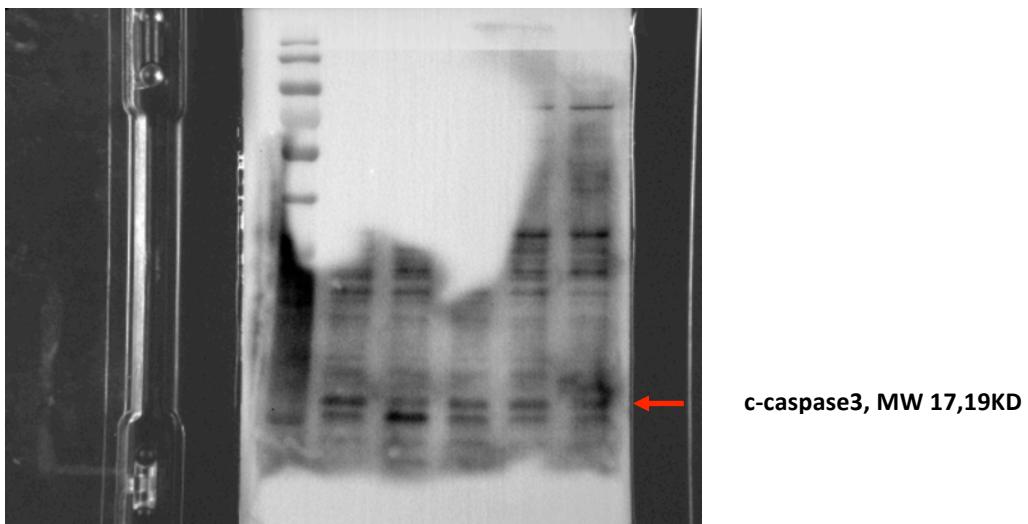
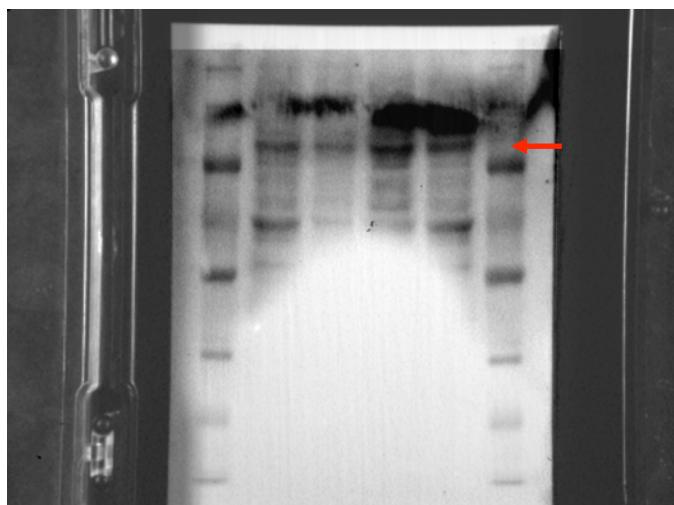
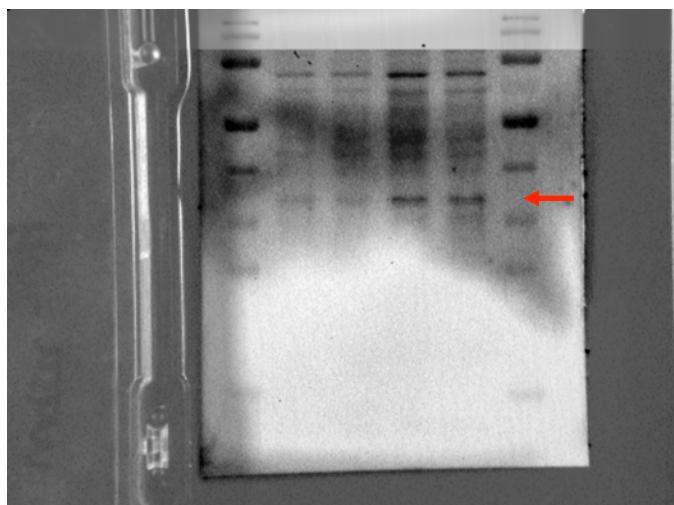


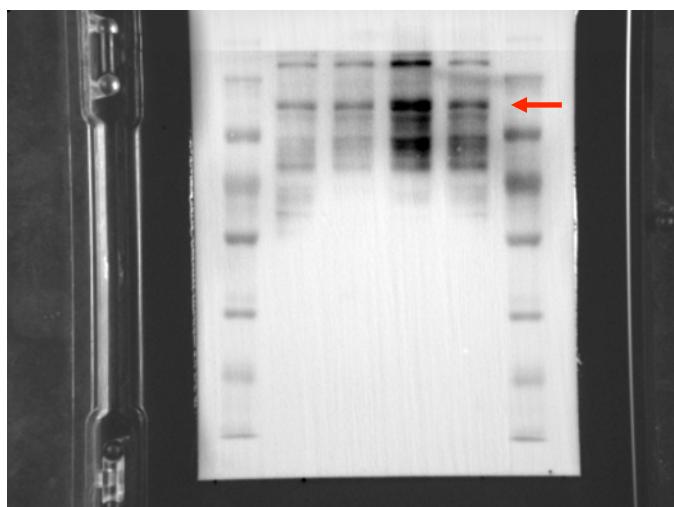
Fig. 7A



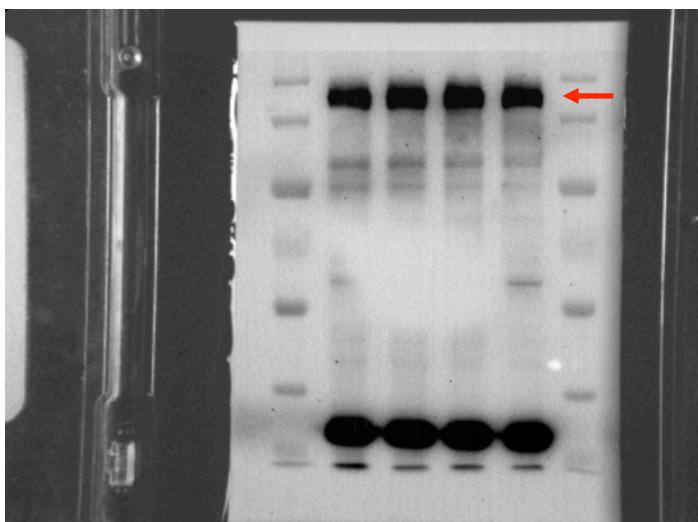
RTN1A, MW 135KD



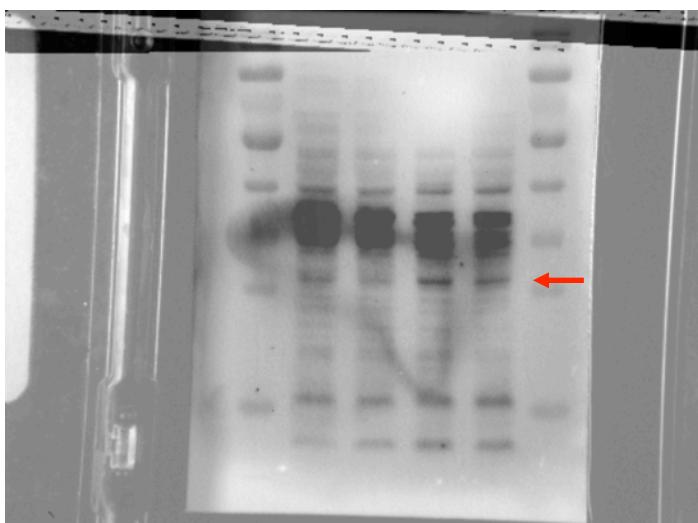
GRP78, MW 78KD



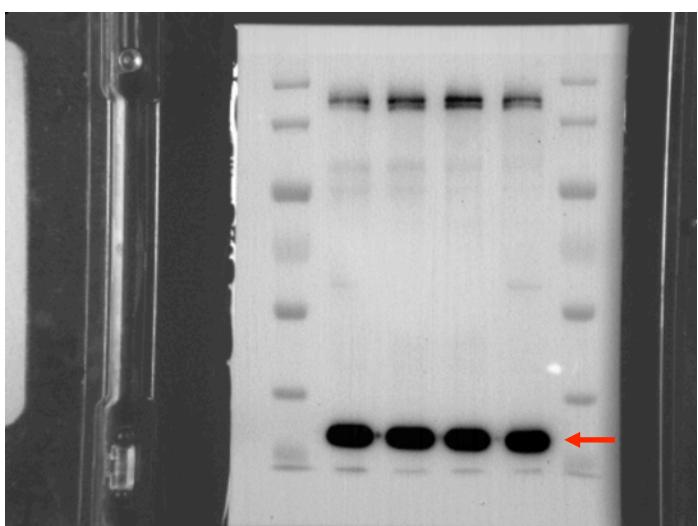
P-PERK, MW 125KD



T-PERK, MW 140KD



CHOP, MW 27KD



GAPDH, MW 36KD

Fig. 7D

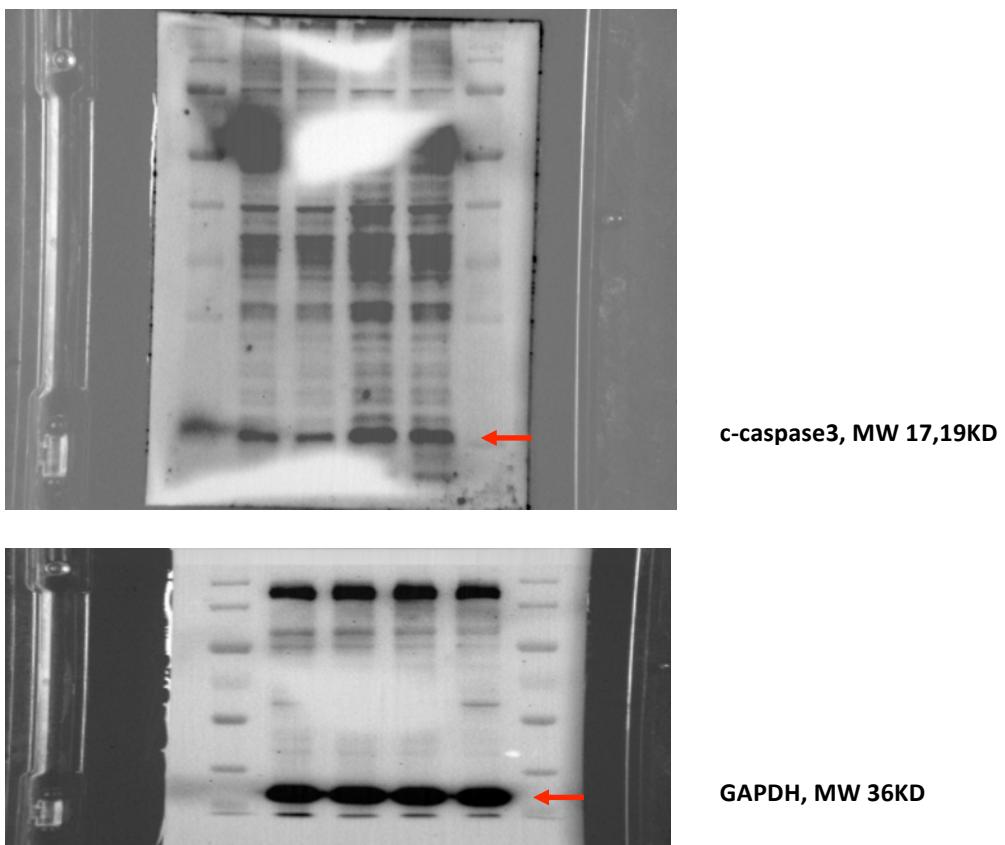
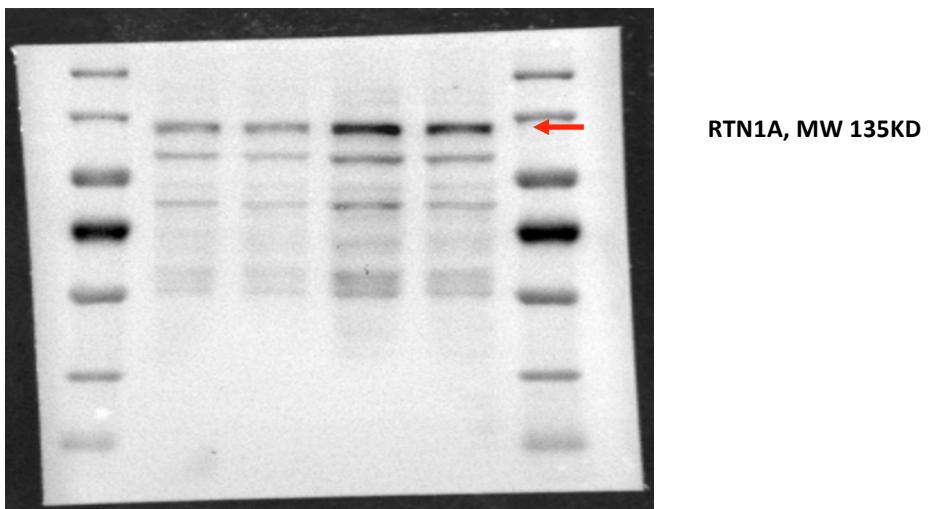
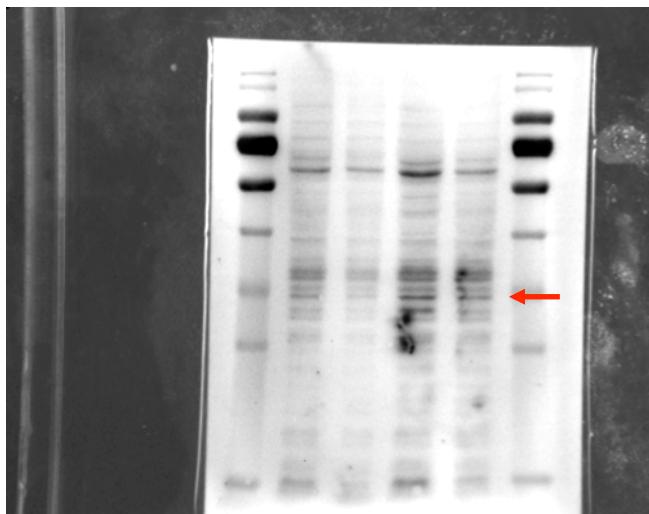
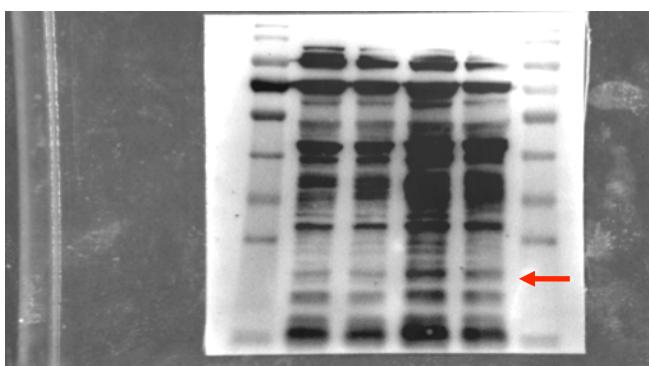


Fig. 8A

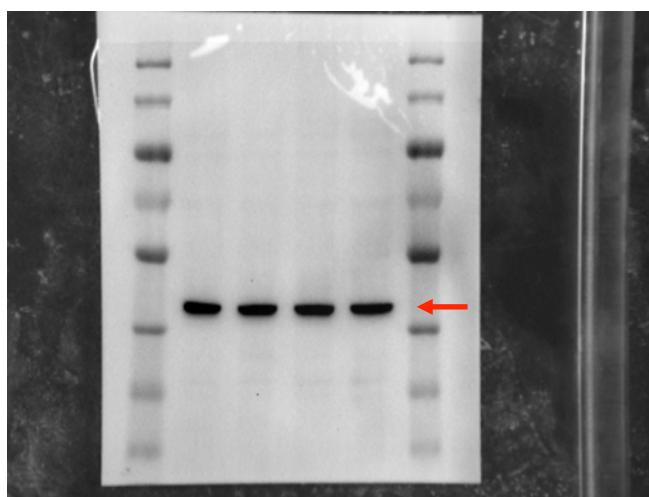




CHOP, MW 27KD



c-caspase3, MW 17,19KD



GAPDH, MW 36KD