Supplementary Information

Functional cargos of exosomes derived from Flk-1$^+$ vascular progenitors enable neurulation and ameliorate embryonic anomalies in diabetic pregnancy

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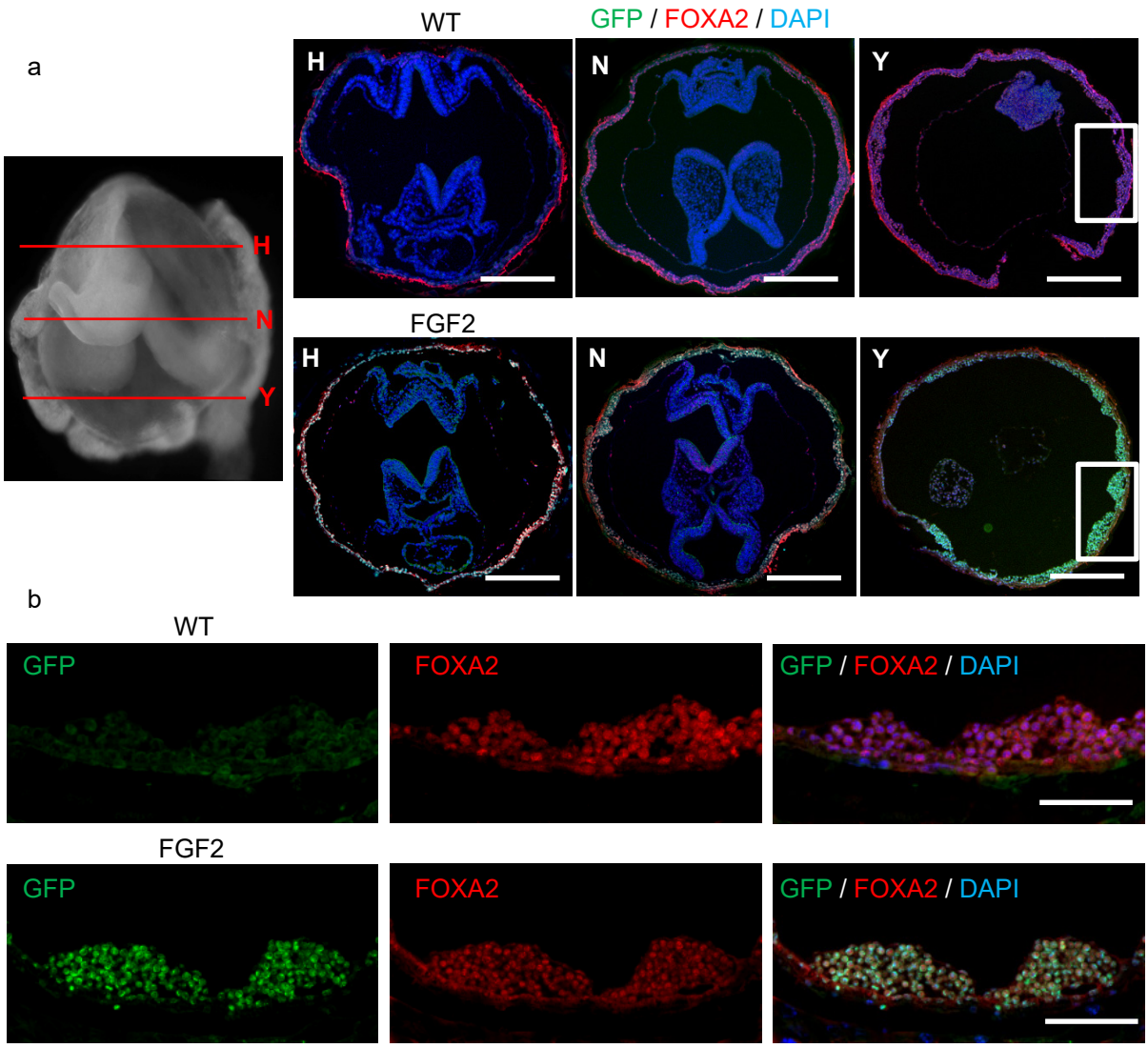
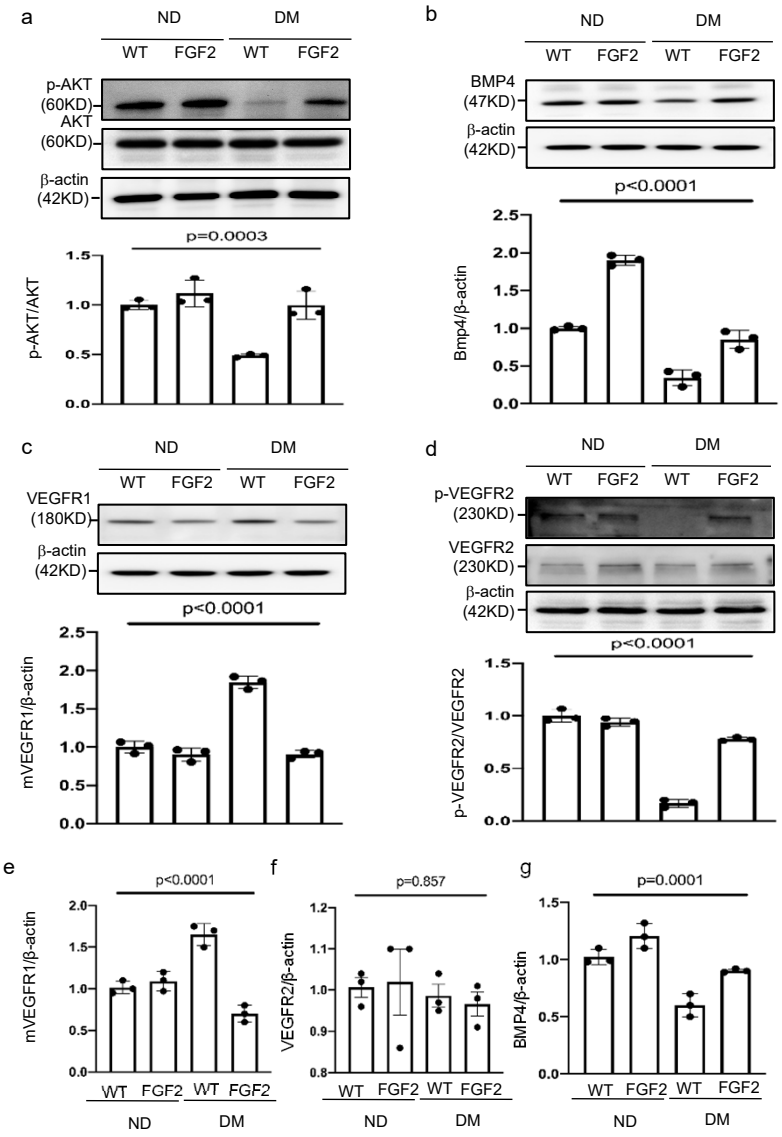
Supplementary Figures and Tables:

Supplementary Figure 1. The transgene Fgf2 is specifically expressed in endoderm cells in the FGF2 Tg embryos.



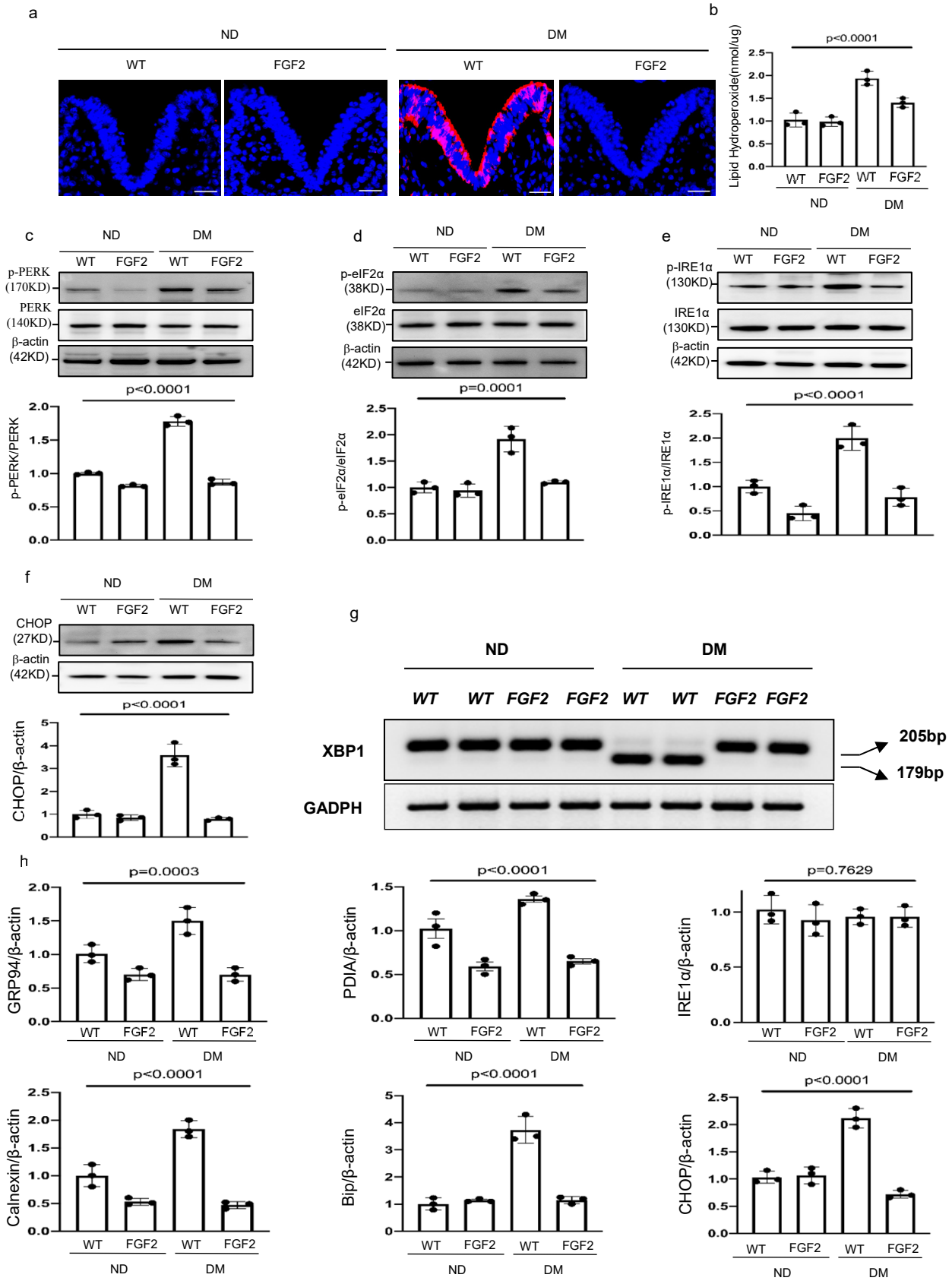
(a) GFP was overlapped with endoderm marker FOXA2 in the yolk sac of E8.5 FGF2 transgenic embryo. Representative sections through Heart (H), Neural fold (N) and York Sac (Y) were displayed. (b) The white squared areas in (a) were enlarged to show detail. Bars in a = 0.25 mm; Bars in b = 0.05 mm.

Supplementary Figure 2. FGF2 transgenic overexpression restores key vascular signaling adversely impacted by maternal diabetes.



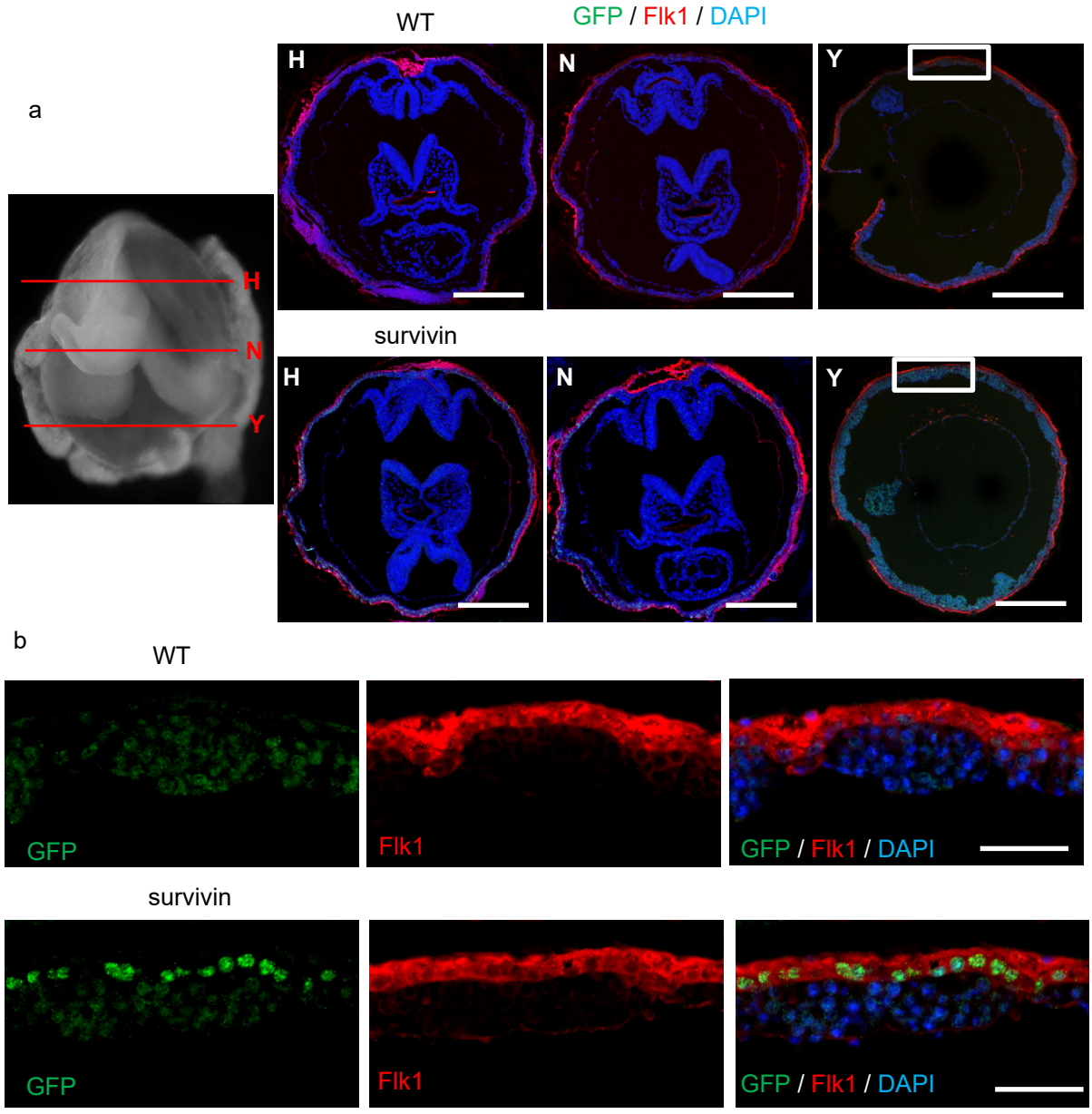
(a to d) Protein levels of (p)-AKT (a), BMP4 (b), VEGFR1 (c) and (p)-VEGFR2 (d) in E8.5 embryos. (e to g) mRNA levels of VEGFR1 (e), VEGFR2 (f) and BMP4 (g) in E8.5 embryos. Experiments were performed using three embryos from three different dams per group (n = 3). * indicates significant difference compared to other groups ($P < 0.05$). ND: nondiabetic; DM: diabetes mellitus; WT: Wild-Type; FGF2: FGF2 transgenic mice.

Supplementary Figure 3. FGF2 overexpression abrogates maternal diabetes-induced cellular stress.



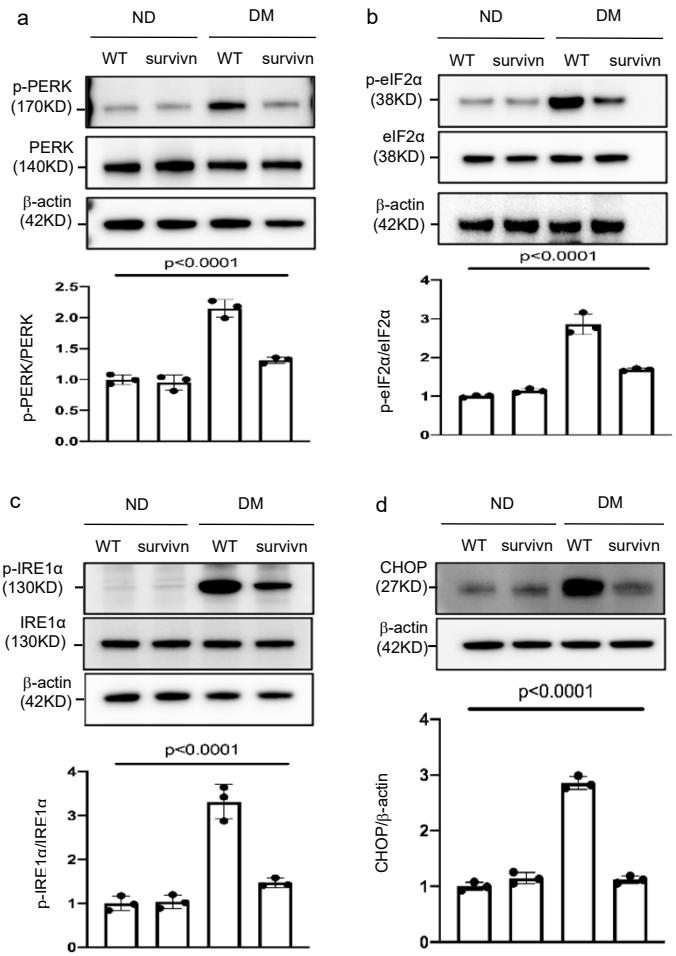
(a) Representative images of DHE staining. Red signals of DHE staining were observed in the V-shaped neuroepithelia of E8.5 embryos exposed to diabetes mellitus (DM). All cell nuclei were stained with DAPI (blue). Bars = 30 μm. (b) Levels of lipid hydroperoxide (LPO) in E8.5 embryos. (c to f) Protein levels of (p)-PERK (c), (p)-eIF2α (d), (p)-IRE1α (e) and CHOP (f) in E8.5 embryos. (g) XBP1 mRNA splicing in E8.5 embryos. Arrows point to the actual size of the bands. (h) mRNA levels of Calnexin, GRP94, PDIA, BiP, IRE1α and CHOP. Experiments were performed using three embryos from three different dams per group (n = 3). * indicates significant difference compared to other groups ($P < 0.05$). ND: nondiabetic; DM: diabetes mellitus; WT: Wild-Type; FGF2: FGF2 transgenic mice.

Supplementary Figure 4. The transgene Survivin is specifically expressed in Flk-1^{+} progenitors in the Survivin Tg embryos.



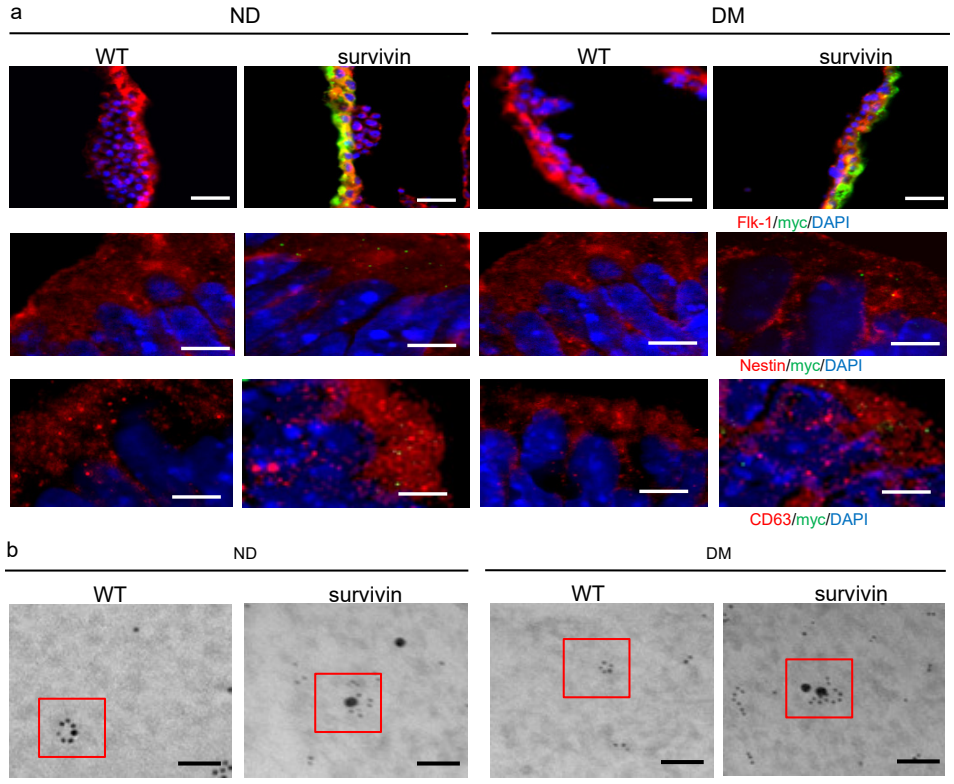
(a) GFP was overlapped with Flk1 in the yolk sac of E8.5 survivin transgenic embryo. Representative sections through the Heart (H), Neural tube (N) and Yolk sac (Y) were displayed. (b) The white square areas in (a) were enlarged to show detail. Bars in a = 0.25 mm; Bars in b = 0.05 mm.

Supplementary Figure 5. Survivin overexpression abrogates maternal diabetes-induced ER stress.



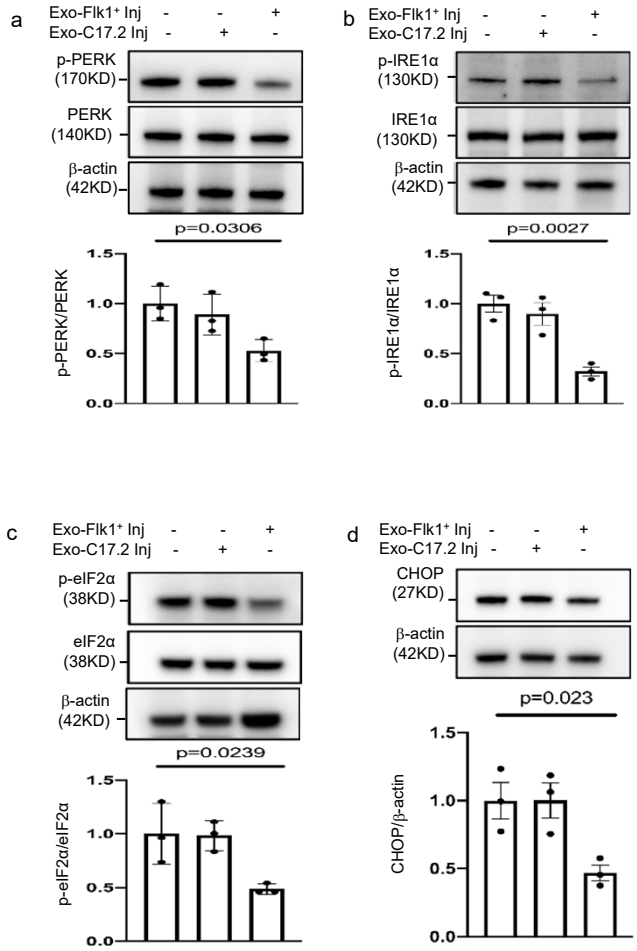
(a to d) Protein levels of (p)-PERK (a), (p)-eIF2α (b), (p)-IRE1α (c) and CHOP (d) in E8.5 embryos. Experiments were performed using three embryos from three different dams per group (n = 3). * indicates a significant difference (P < 0.05) compared to the other groups. ND: nondiabetic; DM: diabetes mellitus; WT: Wild-Type; Survivin: Survivin transgenic mice.

Supplementary Figure 6. Neuroepithelial cells up-take Survivin-contained exosomes derived from Flk-1+ progenitors.



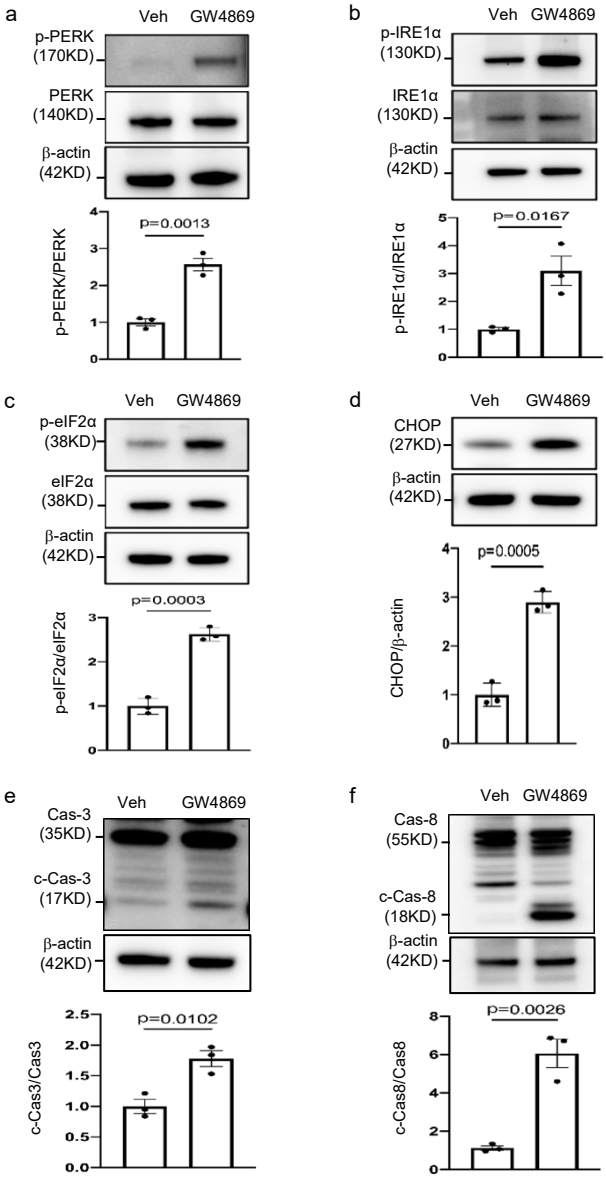
(**a**) Representative imaging of Flk-1 or Nestin (the neural stem cell marker) or CD63 (the exosome marker) (red fluoresce) with myc staining (green) for myc-tagged Survivin the yolk sacs (the top panel) and the neuroepithelia (the middle and the bottom panels) of E8.5 embryos. All cell nuclei were stained with DAPI (Blue). Bars = 30 μm and 10 μm, for the top panel and the low two panels, respectively. (**b**) Representative imaging of electron microscopy with CD63 (small-size silver grains) and myc (large-size silver grains) staining in E8.5 embryonic sections. Bars = 1 μm. Experiments were performed using three embryos from three different dams per group (n = 3). * indicates significant difference compared to other groups ($P < 0.05$). ND: nondiabetic; DM: diabetes mellitus; WT: Wild-Type; Survivin: Survivin transgenic mice.

Supplementary Figure 7. Exosomes from Flk-1$^+$ cells but not from C17.2 neural stem cells abrogates maternal diabetes-induced ER stress.



(**a** to **d**) Protein levels of (p)-PERK (**a**), (p)-IRE1α (**b**), (p)-eIF2α (**c**) and CHOP (**d**) in E8.5 embryos after intra-ammonic injections of exosomes in E8.0 embryos of diabetic dams. The group that did not receive any exosomes were mock injections of vehicle (PBS). Experiments were repeated three times (n = 3). * indicates significant difference compared to other groups ($P < 0.05$).

Supplementary Figure 8. GW4869 mimics maternal diabetes-induced ER stress and apoptosis.



(**a** to **d**) Protein levels of (p)-PERK (**a**), (p)-IRE1α (**b**), (p)-eIF2α (**c**) and CHOP (**d**) in E8.5 embryos after GW4869 injections. (**e**) Protein levels of cleaved caspase 3 after GW4869 injections in E8.5 embryos. (**f**). Protein levels of cleaved caspase 8 after GW4869 injections in E8.5 embryos. Experiments were performed using three embryos from three different dams per group (n = 3). * indicates significant difference compared to other groups ($P < 0.05$). Veh: vehicle (saline) injections.

Supplementary Figure 9: Uncropped Western

Figure 1

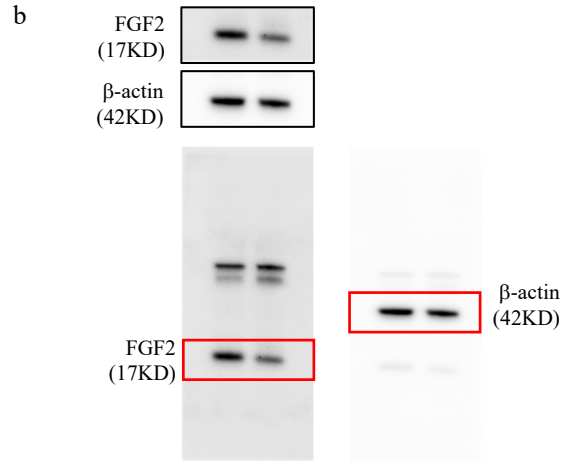


Figure 3

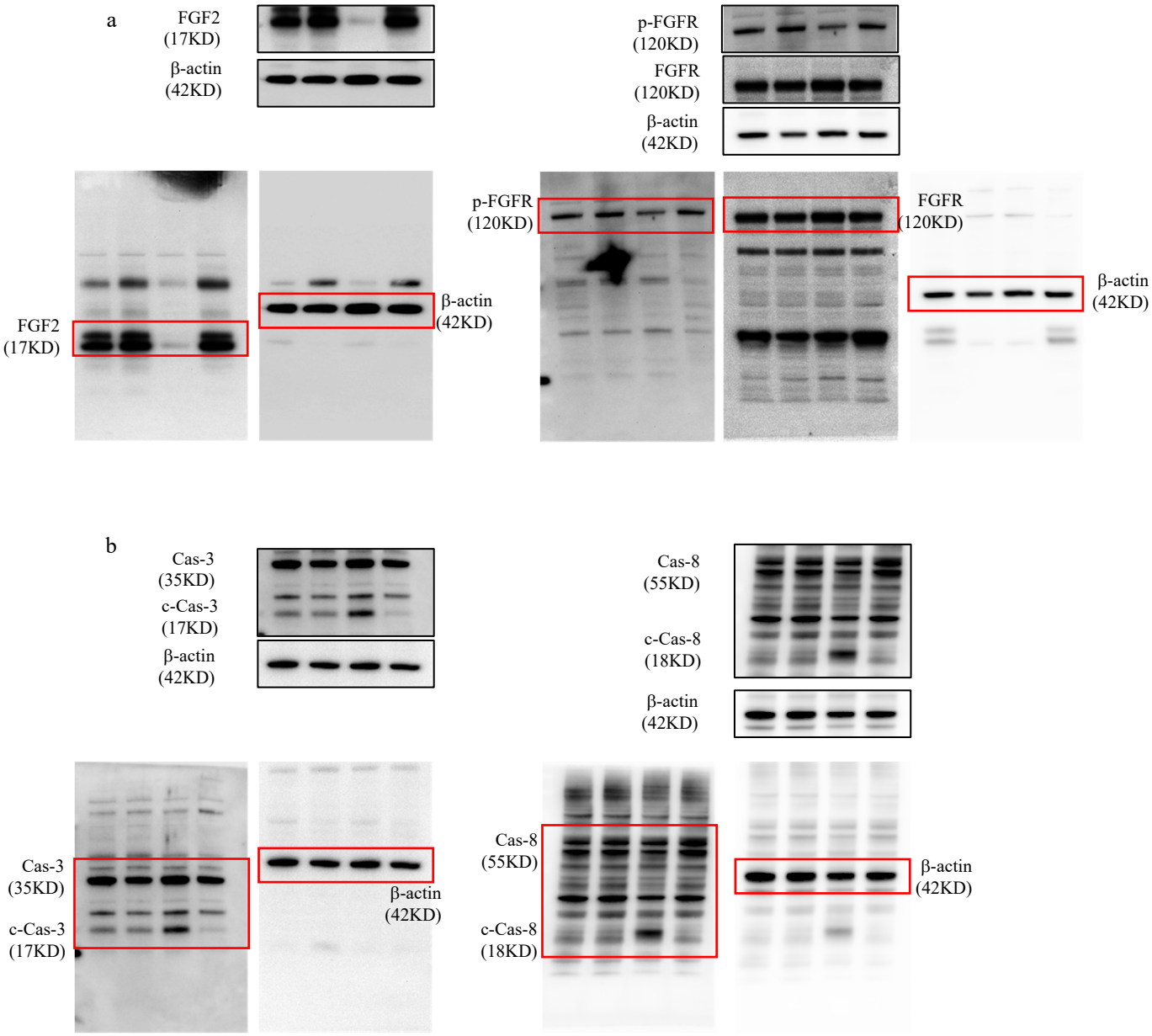

a
FGF2
(17KD)
β-actin
(42KD)
p-FGFR
(120KD)
FGFR
(120KD)
β-actin
(42KD)
FGF2
(17KD)
β-actin
(42KD)
p-FGFR
(120KD)
FGFR
(120KD)
β-actin
(42KD)
b
Cas-3
(35KD)
c-Cas-3
(17KD)
β-actin
(42KD)
Cas-8
(55KD)
c-Cas-8
(18KD)
β-actin
(42KD)
Cas-3
(35KD)
c-Cas-3
(17KD)
β-actin
(42KD)
Cas-8
(55KD)
c-Cas-8
(18KD)
β-actin
(42KD)

Figure 4

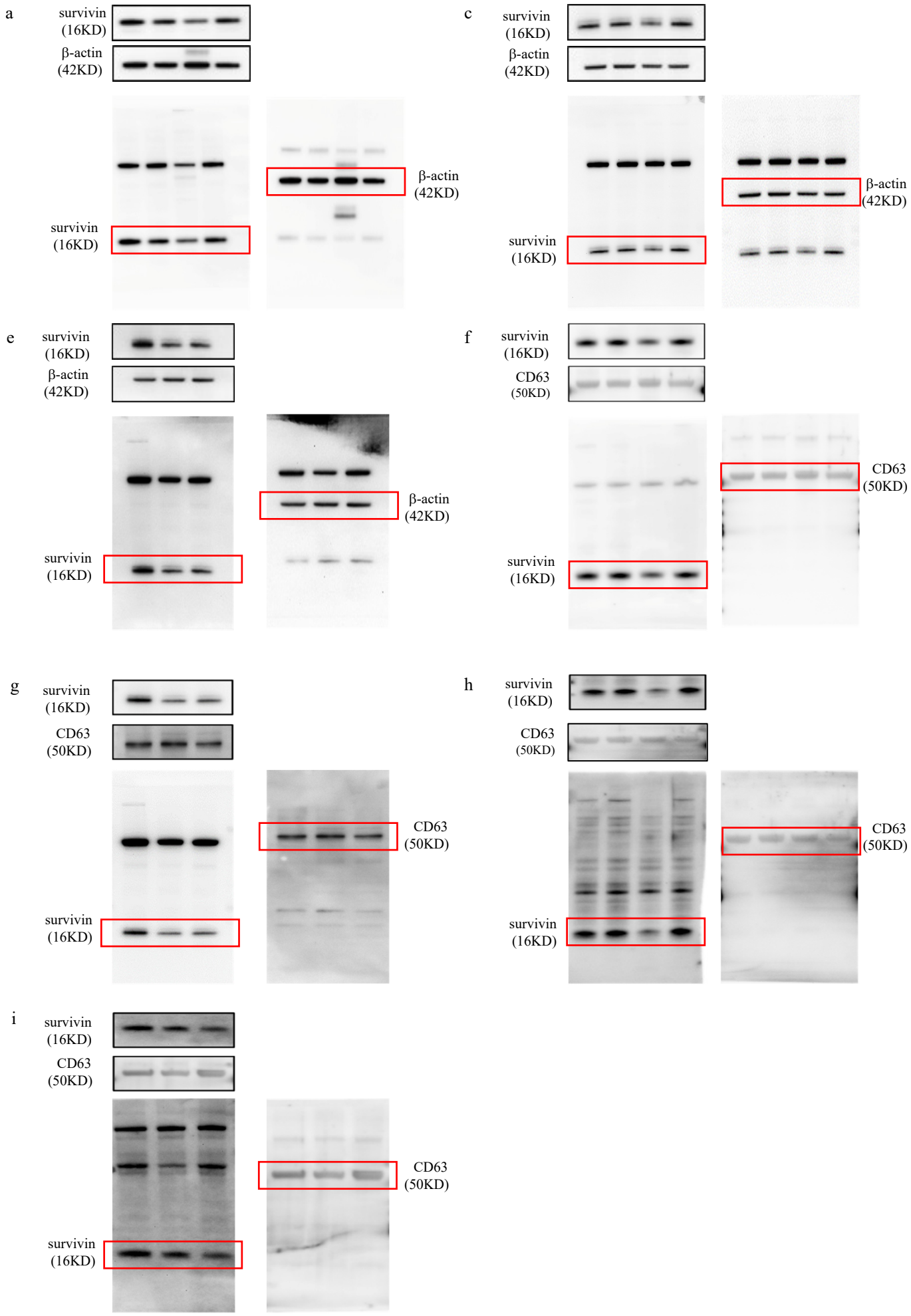

a
survivin (16KD)
β-actin (42KD)
survivin (16KD)
β-actin (42KD)
c
survivin (16KD)
β-actin (42KD)
survivin (16KD)
β-actin (42KD)
e
survivin (16KD)
β-actin (42KD)
survivin (16KD)
β-actin (42KD)
f
survivin (16KD)
CD63 (50KD)
survivin (16KD)
CD63 (50KD)
g
survivin (16KD)
CD63 (50KD)
survivin (16KD)
CD63 (50KD)
h
survivin (16KD)
CD63 (50KD)
survivin (16KD)
CD63 (50KD)
i
survivin (16KD)
CD63 (50KD)
survivin (16KD)
CD63 (50KD)

Figure 5

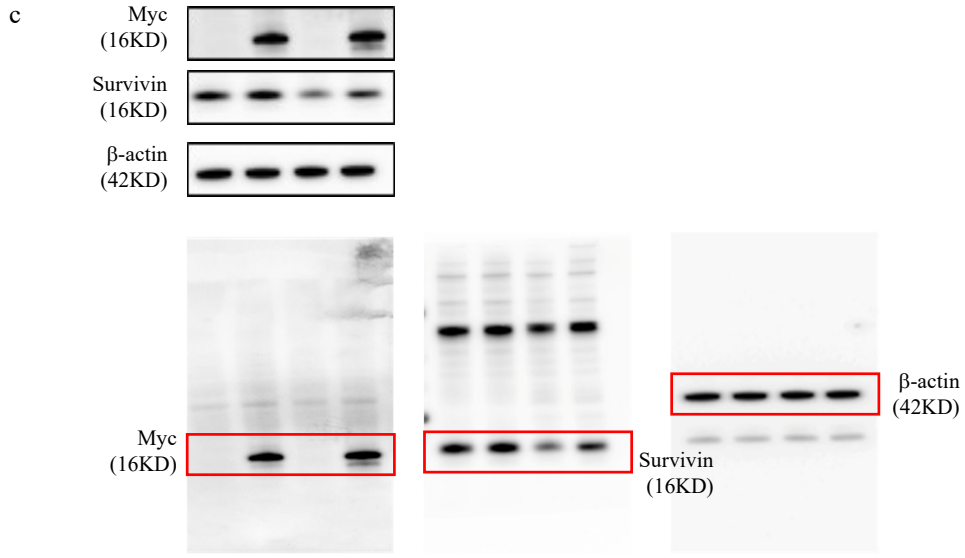

c
Myc
(16KD)
Survivin
(16KD)
β-actin
(42KD)
Myc
(16KD)
Survivin
(16KD)
β-actin
(42KD)

Figure 6

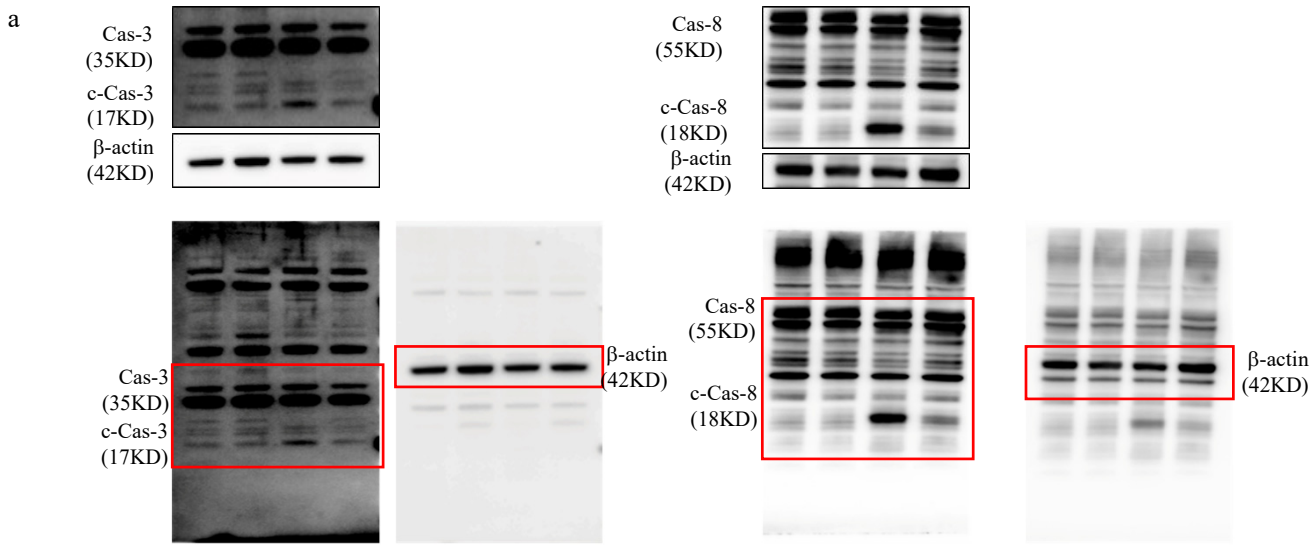
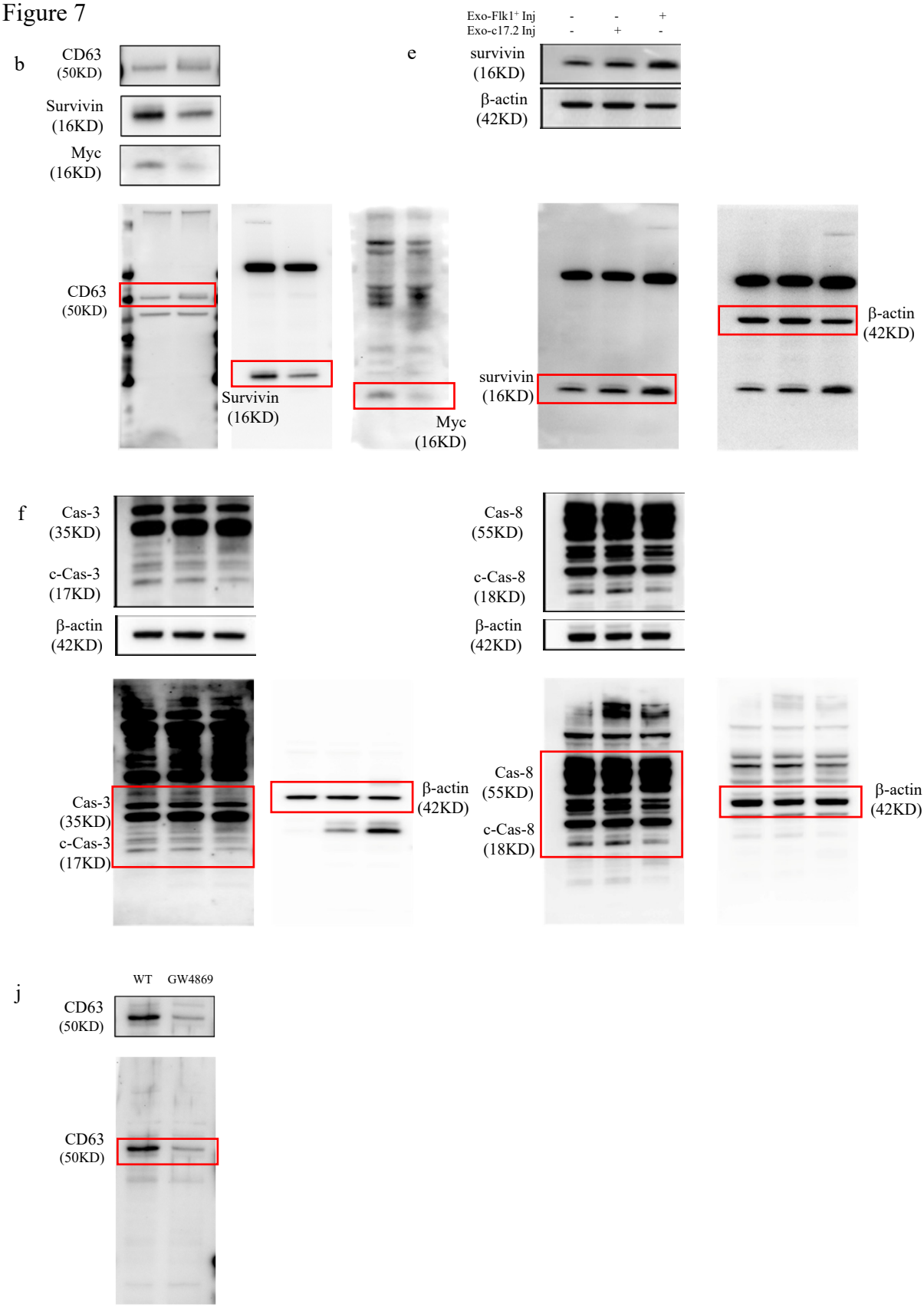
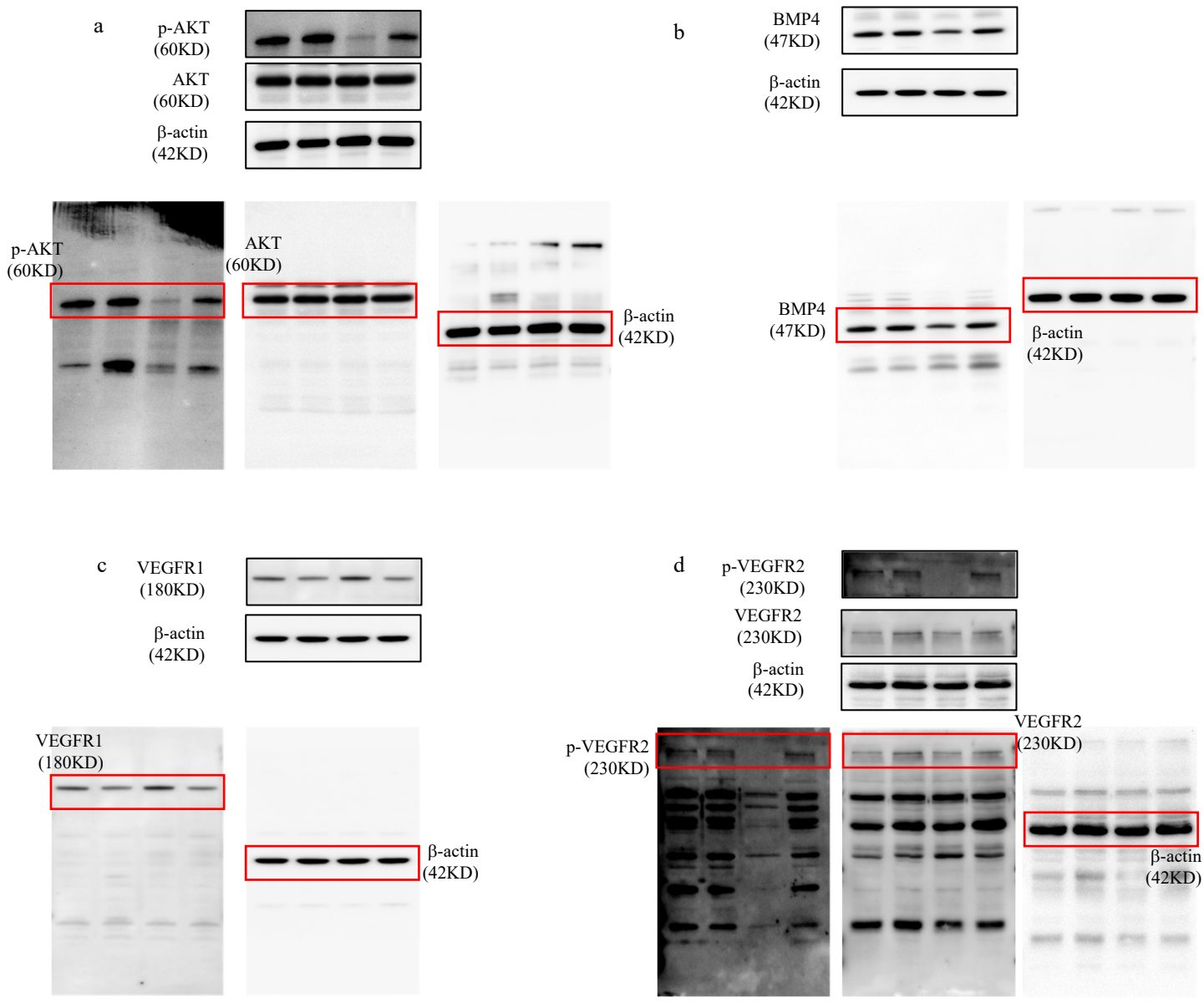

a
Cas-3
(35KD)
c-Cas-3
(17KD)
β-actin
(42KD)
Cas-8
(55KD)
c-Cas-8
(18KD)
β-actin
(42KD)
Cas-3
(35KD)
c-Cas-3
(17KD)
β-actin
(42KD)
Cas-8
(55KD)
c-Cas-8
(18KD)
β-actin
(42KD)

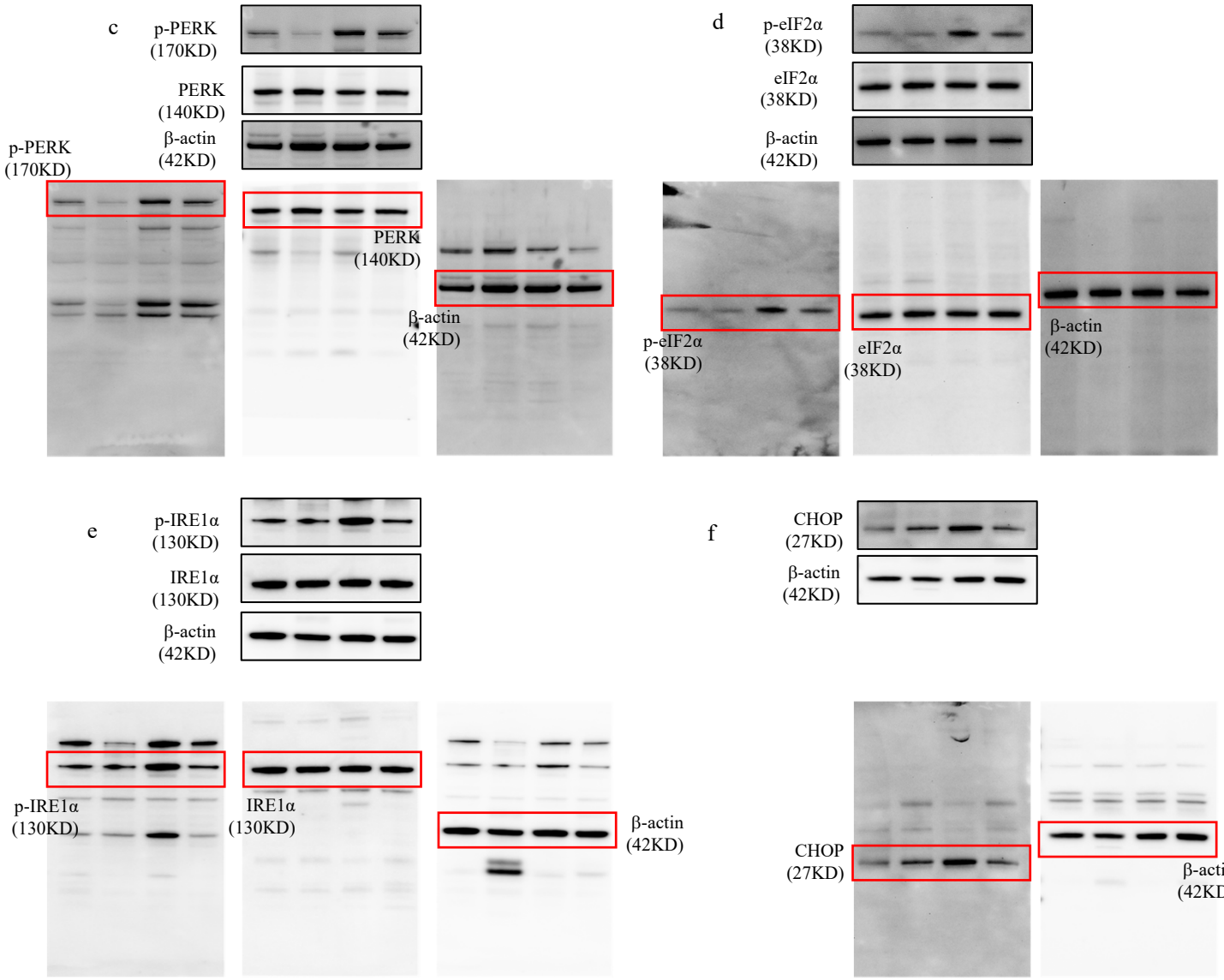
Figure 7


b
CD63
(50KD)
Survivin
(16KD)
Myc
(16KD)
CD63
(50KD)
Survivin
(16KD)
Myc
(16KD)
e
Exo-Flk1+ Inj - - +
Exo-c17.2 Inj - + -
survivin
(16KD)
β-actin
(42KD)
survivin
(16KD)
β-actin
(42KD)
f
Cas-3
(35KD)
c-Cas-3
(17KD)
β-actin
(42KD)
Cas-3
(35KD)
c-Cas-3
(17KD)
β-actin
(42KD)
Cas-8
(55KD)
c-Cas-8
(18KD)
β-actin
(42KD)
Cas-8
(55KD)
c-Cas-8
(18KD)
β-actin
(42KD)
j
WT GW4869
CD63
(50KD)
CD63
(50KD)

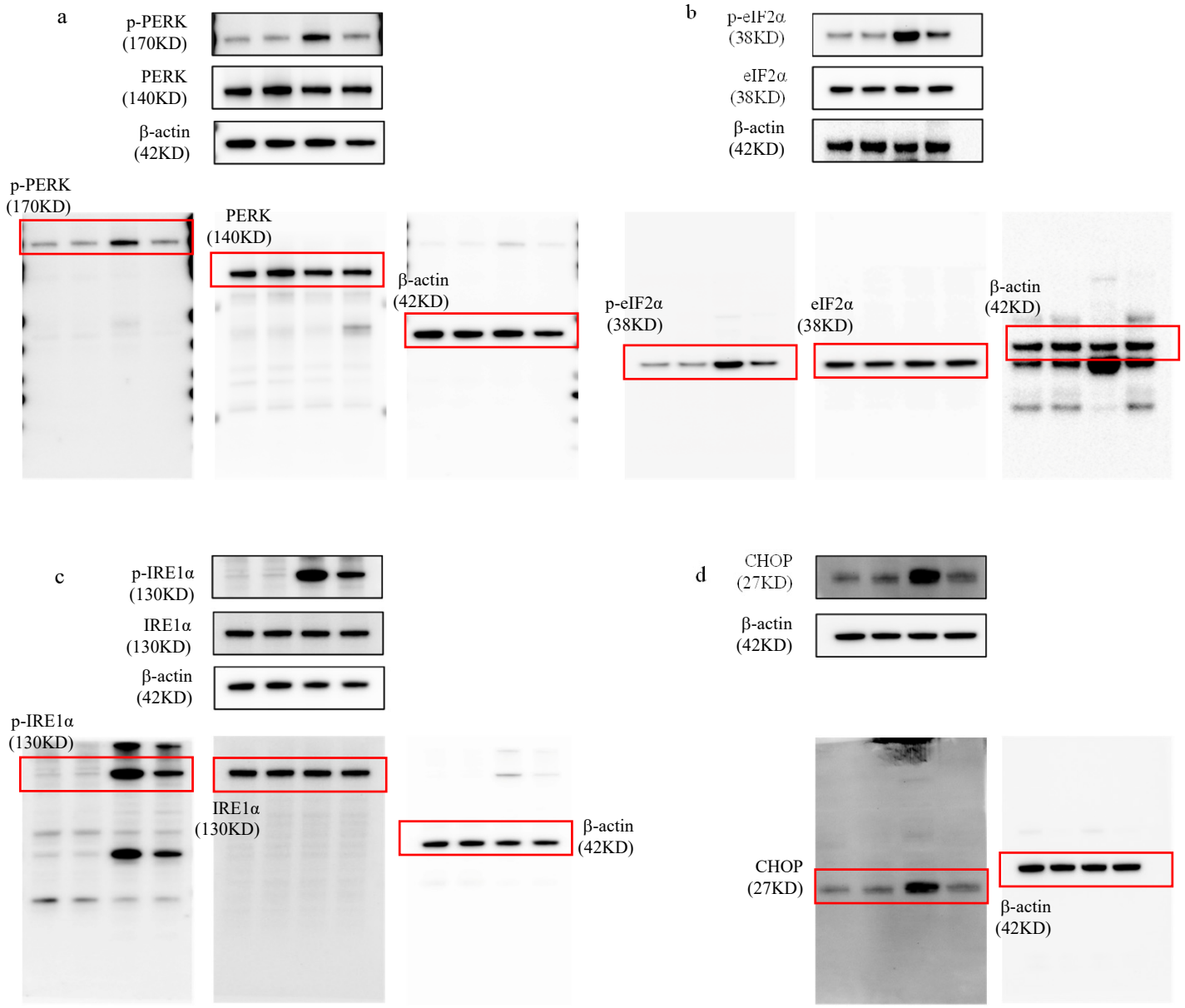
Supplementary Figure 2



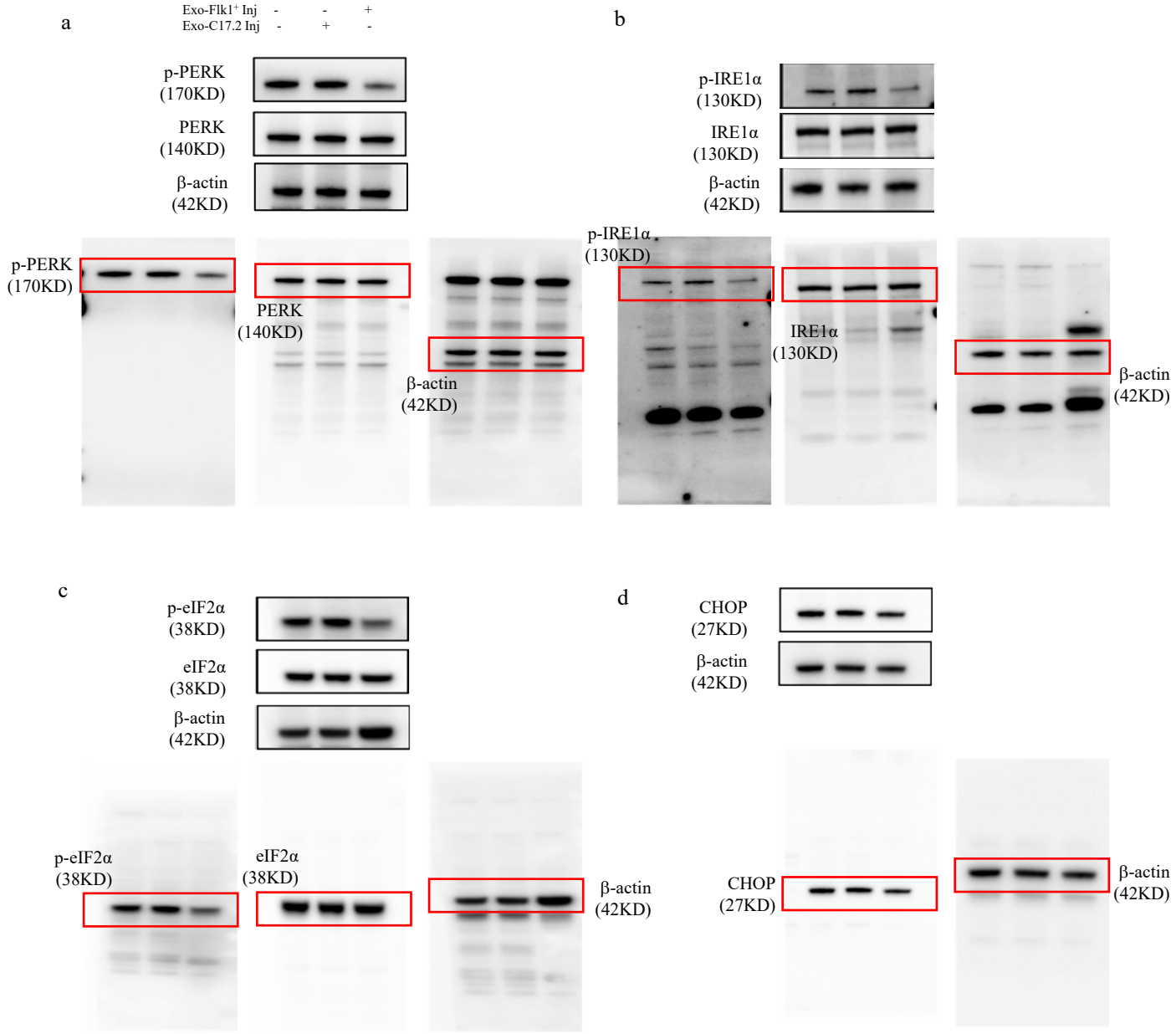
Supplementary Figure 3



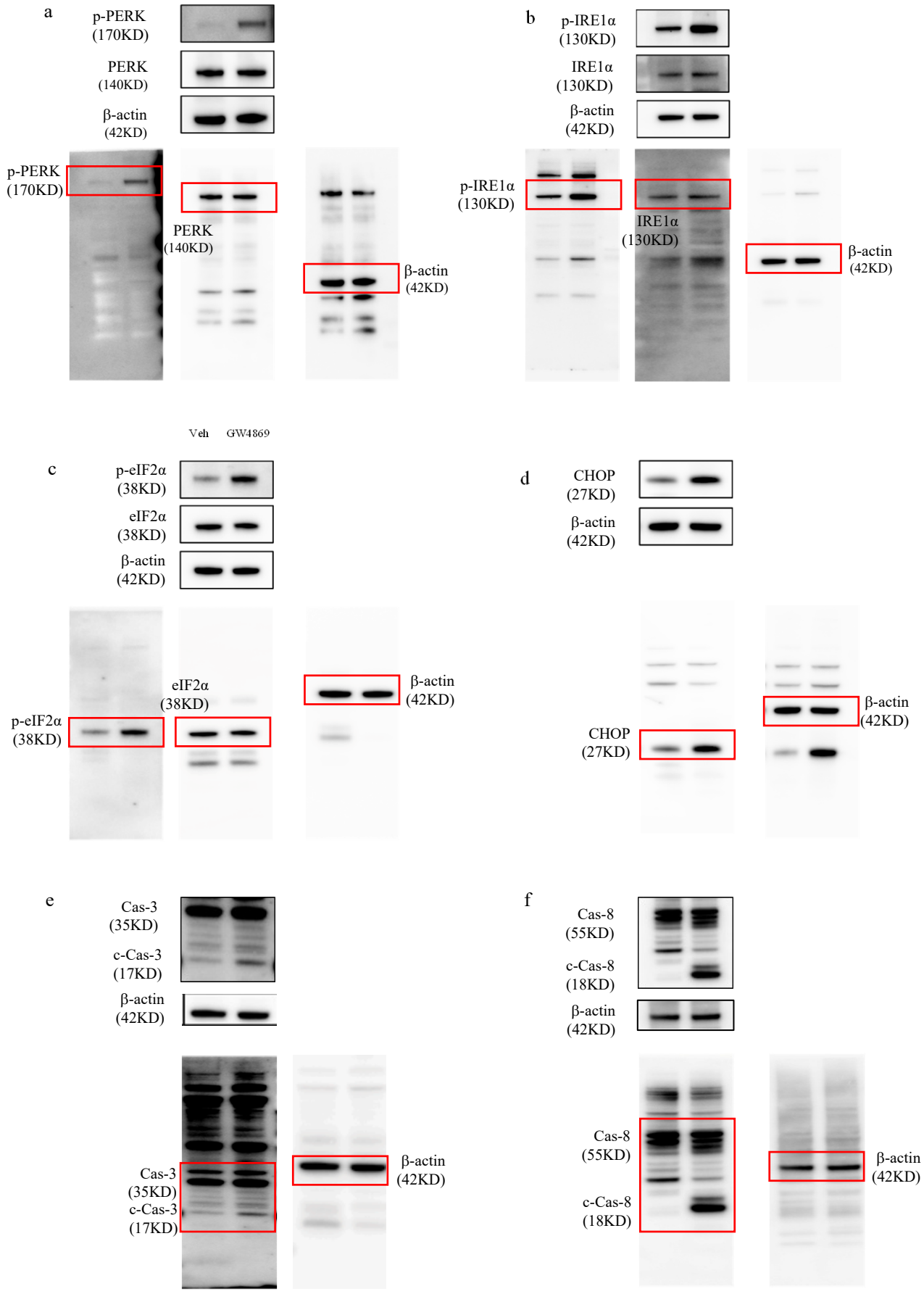
Supplementary Figure 5



Supplementary Figure 7



Supplementary Figure 8



Supplementary Table 1. FGF2 overexpression during vasculogenesis ameliorates maternal diabetes- induced NTDs

	Experimental group	Glucose level (mg/dl)	Genotype	Embryos	NTD Embryos (NTD rate)
ND	FGF2-Tg male x WT female (17 litters)	136.7±30.3	WT	56	0(0.0%)
			FGF2-Tg	58	1(1.7%)
DM	FGF2-Tg male x WT female (17 litters)	388.4±21.4	WT	52	12(23.1%) *
			FGF2-Tg	53	3(5.7%)

NTD, neural tube defect; ND: nondiabetic; DM: diabetes mellitus; WT: Wild-Type; Tg: transgenic. * indicates significant difference compared to other groups ($P < 0.05$) analyzed by the Fisher's Exact test.

Supplementary Table 2. Survivin overexpression during vasculogenesis ameliorates maternal diabetes-induced NTDs

	Experimental group	Glucose level (mg/dl)	Genotype	Embryos	NTD Embryos (NTD rate)
ND	Survivin-Tg male x WT female (11 litters)	123.9±28.5	WT	47	0(0.0%)
			Survivin-Tg	42	0(0.0%)
DM	Survivin-Tg male x WT female (17 litters)	426.8±26.7	WT	63	17(26.98%) *
			Survivin-Tg	61	7(11.48%)

NTD, neural tube defect; ND: nondiabetic; DM: diabetes mellitus; WT: Wild-Type; Tg: transgenic. * indicates significant difference compared to other groups ($P < 0.05$) analyzed by the Fisher's Exact test.

Supplementary Table 3. Microinjecting exosomes to embryos reduce maternal diabetes-induced NTDs

	Experimental group	Glucose level (mg/dl)	Genotype	Embryos	NTD Embryos (NTD rate)
DM	WT male x WT female (20 litters)	416.15±53.1	Non-injection	73	17(23.29%)
			injection	26	1(3.85%) *
			Con-injection	37	7(18.92%)

NTD, neural tube defect; DM: diabetes mellitus; WT: Wild-Type. * Indicates significant difference ($P < 0.05$) when compared to other groups analyzed by the Fisher's Exact test. 7 embryos absorbed after injection, 3 embryos absorbed with non-injection.

Supplementary Table 4. An exosome inhibitor induces NTDs

	Experimental group	Glucose level (mg/dl)	Genotype	Embryos	NTD Embryos (NTD rate)
ND	WT male x WT female (15 litters)	128.6±31.6	GW4869	96	7(7.29%) *
	WT male x WT female (8 litters)	121.38±8.78	vehicle	62	0(0.0%)

NTD, neural tube defect; ND: nondiabetic; WT: Wild-Type. * Indicates significant difference ($P < 0.05$) when compared to other groups analyzed by the Fisher's Exact test.

Supplementary Table 5. Antibody information and other resource information

Antibodies name	Antibody sources	Identifier
FGF2	Millipore	05-118
p-FGFR	Invitrogen	44-1140G
FGFR	Santa Cruz biotechnology	9740s
p-AKT	Cell Signaling Technology	4060s
AKT	Cell Signaling Technology	9272s
BMP4	Abcam	ab39973
VEGFR1	Invitrogen	13687-1-AP
p-IRE1a	Invitrogen	PA1-16927
IRE1a	Cell Signaling Technology	3294
p-PERK	Cell Signaling Technology	3179
PERK	Cell Signaling Technology	3192
p-eIF2a	Cell Signaling Technology	3597
eIF2a	Cell Signaling Technology	2103
CHOP	Cell Signaling Technology	5554
Caspase8	Millipore	AB1879
Caspase 3	Millipore	AB1899
CD31	Abcam	ab28364
FLK1	Santa Cruz	sc6251
nestin	Invitrogen	MA1-110
survivin	Cell Signaling Technology	2808s
myc	Cell Signaling Technology	2272s
CD63	Santa Cruz Biotechnology	sc-5275
DAPI	Invitrogen	D1306

Alexa Fluor 488-conjugated donkey anti-mouse IgG secondary antibody	Invitrogen	A-21202
Alexa Fluor 488-conjugated donkey anti-rabbit IgG secondary antibody	Invitrogen	A-21206
Alexa Fluor 594-conjugated donkey anti-mouse IgG secondary antibody	Invitrogen	A-21203
Alexa Fluor 594-conjugated donkey anti-rabbit IgG secondary antibody	Invitrogen	A-21207
β-actin	Abcam	ab8224

Supplementary Table 6. PCR Primers

Primers name	Primer sequences (5'-3')	
Fgf2	Forward primer	GCGACCCACACGTCAAACTA
	Reverse primer	TCCCTTGATAGACACAACTCCTC
Fgfr	Forward primer	ACTCTGCGCTGGTTGAAAAAT
	Reverse primer	GGTGGCATAGCGAACCTTGTA
BiP	Forward primer	ACTTGGGGACCACCTATTCCT
	Reverse primer	ATCGCCAATCAGACGCTCC
CHOP	Forward primer	CGGAACCTGAGGAGAGAGTG
	Reverse primer	CTGTCAGCCAAGCTAGGGAC
Calnexin	Forward primer	ATGGAAGGGAAGTGGTTACTGT
	Reverse primer	GCTTTGTAGGTGACCTTTGGAG
IRE1α	Forward primer	ACACCGACCACCGTATCTCA
	Reverse primer	CTCAGGATAATGGTAGCCATGTC
PDIA	Forward primer	CGCCTCCGATGTGTTGGA
	Reverse primer	CAGTGCAATCCACCTTTGCTAA
GRP94	Forward primer	TCGTCAGAGCTGATGATGAAGT
	Reverse primer	GCGTTTAACCCATCCAACTGAAT
BMP4	Forward primer	TTCCTGGTAACCGAATGCTGA
	Reverse primer	CCTGAATCTCGGCGACTTTTT
VEGFR1	Forward primer	CCACCTCTCTATCCGCTGG
	Reverse primer	ACCAATGTGCTAACCGTCTTATT
VEGFR2	Forward primer	TTTGGCAAATACAACCCTTCAGA
	Reverse primer	GCAGAAGATACTGTCACCACC
survivin	Forward primer	CTACCGAGAACGAGCCTGATT

	Reverse primer	AGCCTTCCAATTCCTTAAAGCAG
β-Actin	Forward primer	GAACCAGGAGTTAAGAACACG
	Reverse primer	AGGCAACAGTGTCAGAGTCC