

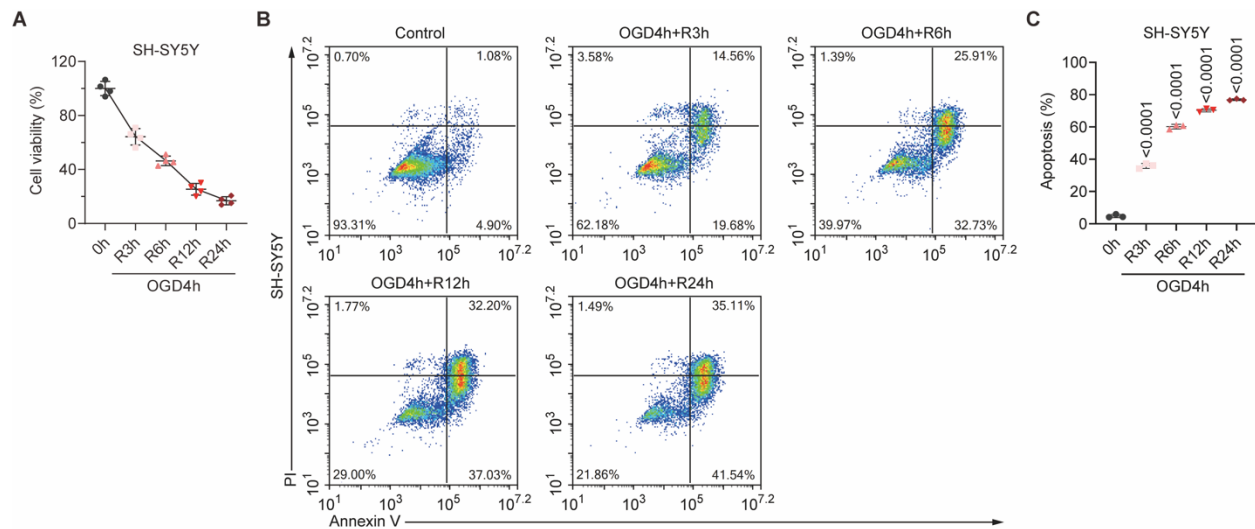


Figure S1. Prolonged reperfusion time led to decreased viability and heightened apoptosis in OGD-treated SH-SY5Y cells. (A) MTT staining results demonstrated a negative correlation between longer reperfusion time and the viability of SH-SY5Y OGD/R cells. (B) Annexin V/PI staining outcomes indicated that extended reperfusion time resulted in increased apoptosis of SH-SY5Y OGD/R cells. (C) Quantification of the Annexin V/PI staining results.

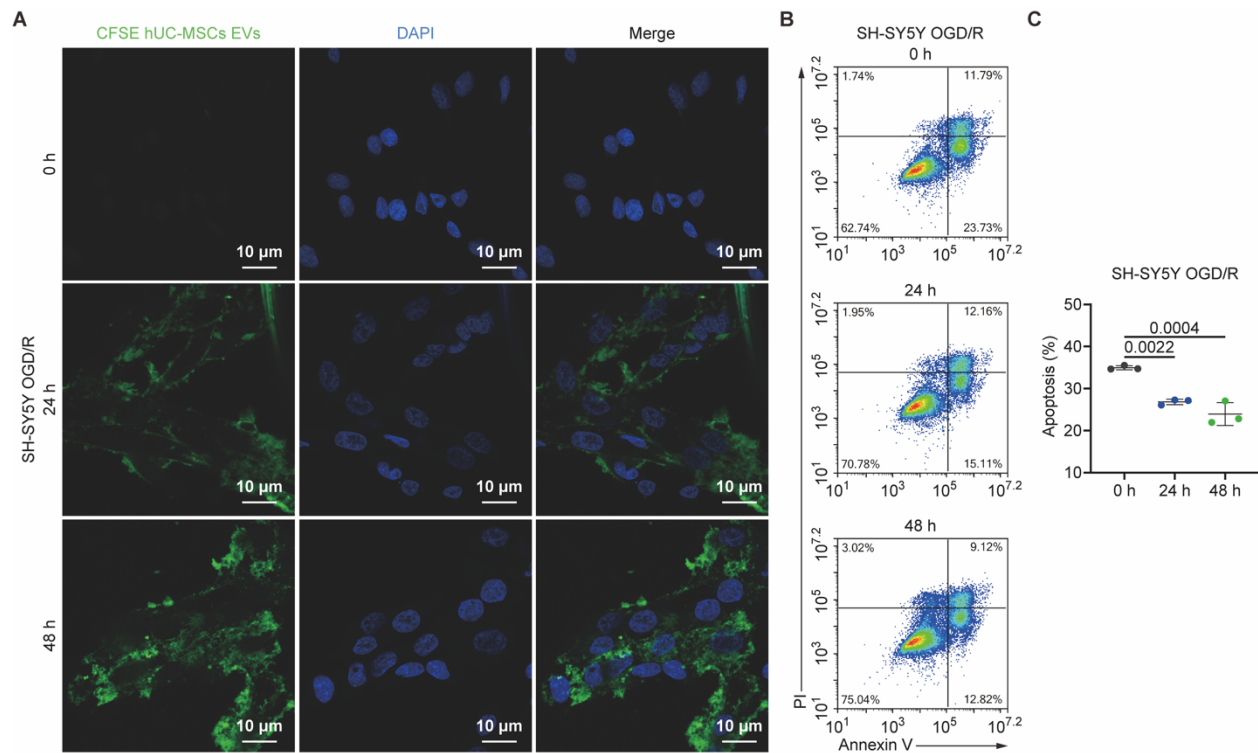


Figure S2. hUC-MSC-EVs can be internalized by SH-SY5Y OGD/R. (A) CFSE-labeled EV tracing assay results indicated that hUC-MSC-EVs can be efficiently absorbed by SH-SY5Y OGD/R. (B) Annexin V/PI staining data revealed that the anti-apoptotic function of hUC-MSC-EVs on cocultured SH-SY5Y OGD/R cells can last at least 48 hours. (C) Quantification of the Annexin V/PI staining data.

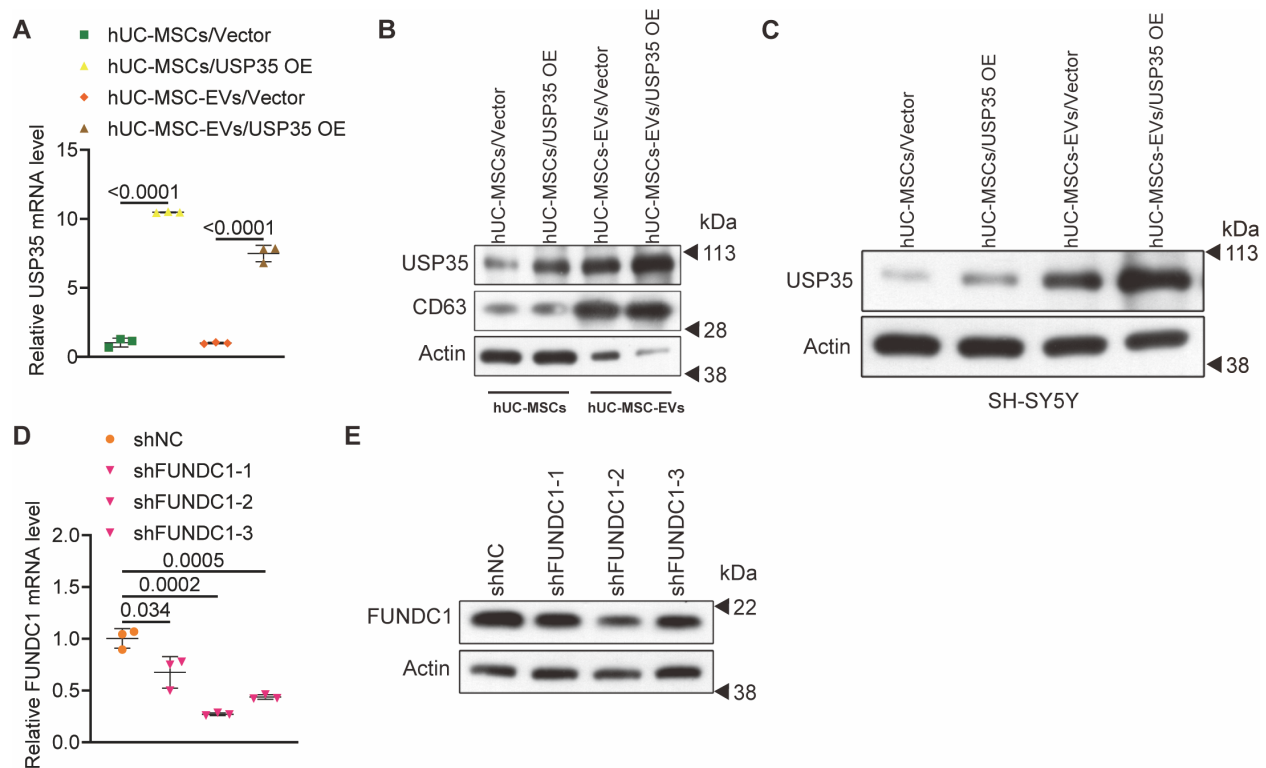


Figure S3. qPCR and Western blot results confirmed USP35 overexpression in hUC-MSC, hUC-MSC-EV, and cocultured SH-SY5Y cells and the efficiency of FUNDC1 knockdown. (A, B) qPCR (A) and Western blot data (B) demonstrated a significant elevation in USP35 expression in hUC-MSCs and their secreted EVs upon USP35 overexpression. (C) Western blot data showed the increased USP35 protein levels in SH-SY5Y cells treated with USP35-overexpressing hUC-MSCs and hUC-MSC-EVs. (D, E) qPCR (D) and Western blot results (E) indicated an effective reduction in FUNDC1 mRNA levels with all three shRNAs targeting FUNDC1, with shFUND1-2 exhibiting the highest efficiency.

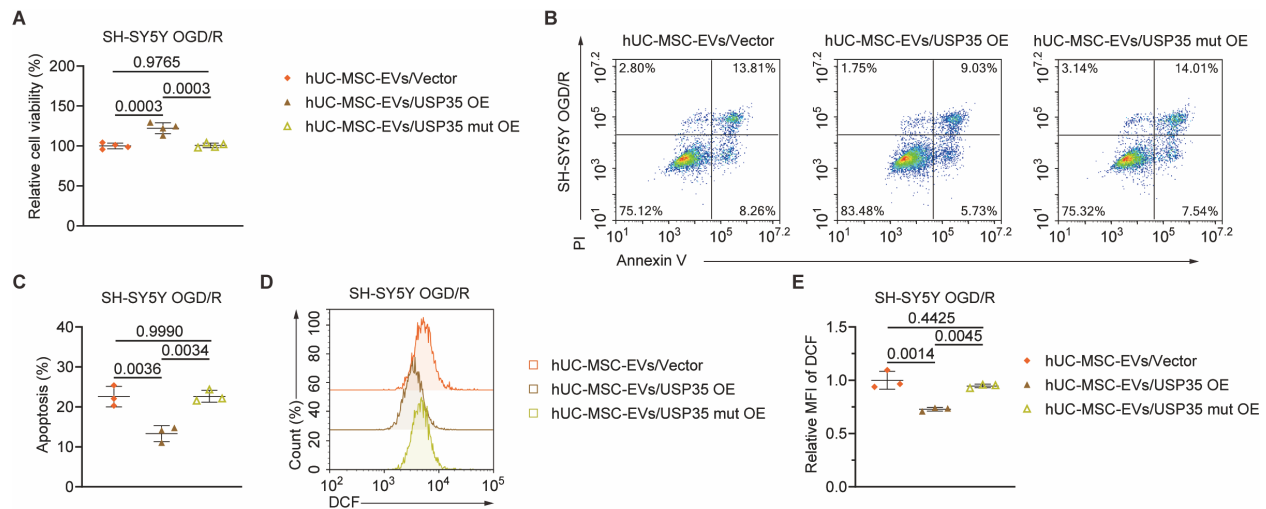


Figure S4. Overexpression of USP35 with deficient catalytic domain had minimal impact on the neuroprotective of hUC-MSC-EVs. (A) MTT staining results demonstrated that hUC-MSC-EVs overexpressing mutant USP35 have a similar effect on the viability of SH-SY5Y OGD/R cells as control hUC-MSC-EVs. (B) Annexin V/PI staining showed that the overexpression of mutant USP35 failed to enhance the anti-apoptotic effect of hUC-MSC-EVs on SH-SY5Y OGD/R cells. (C) Quantification of the Annexin V/PI staining results. (D) DCF staining revealed that control hUC-MSC-EVs and mutant USP35-overexpressing hUC-MSC-EVs had comparable effects on the ROS levels of cocultured SH-SY5Y OGD/R cells. (H) Quantification of the DCF staining results.

#1 hUC-MSCs
#2 hUC-MSC-EVs
#3 hUC-MSC+GW4869-EVs

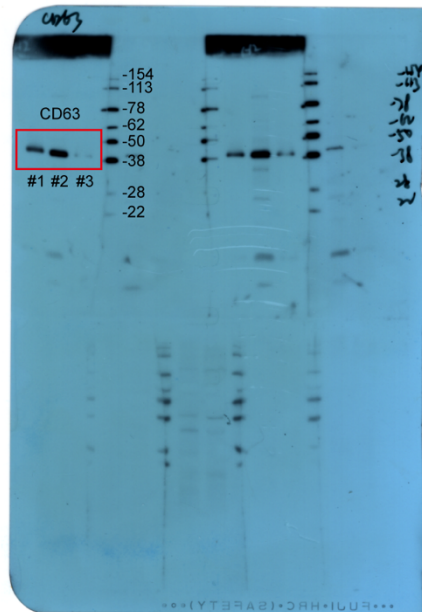


Fig S5. Uncropped blot results for Fig. 1D

#1 Mock
 #2 hUC-MSCs
 #3 hUC-MSCs+GW4869
 #4 hUC-MSC-EVs

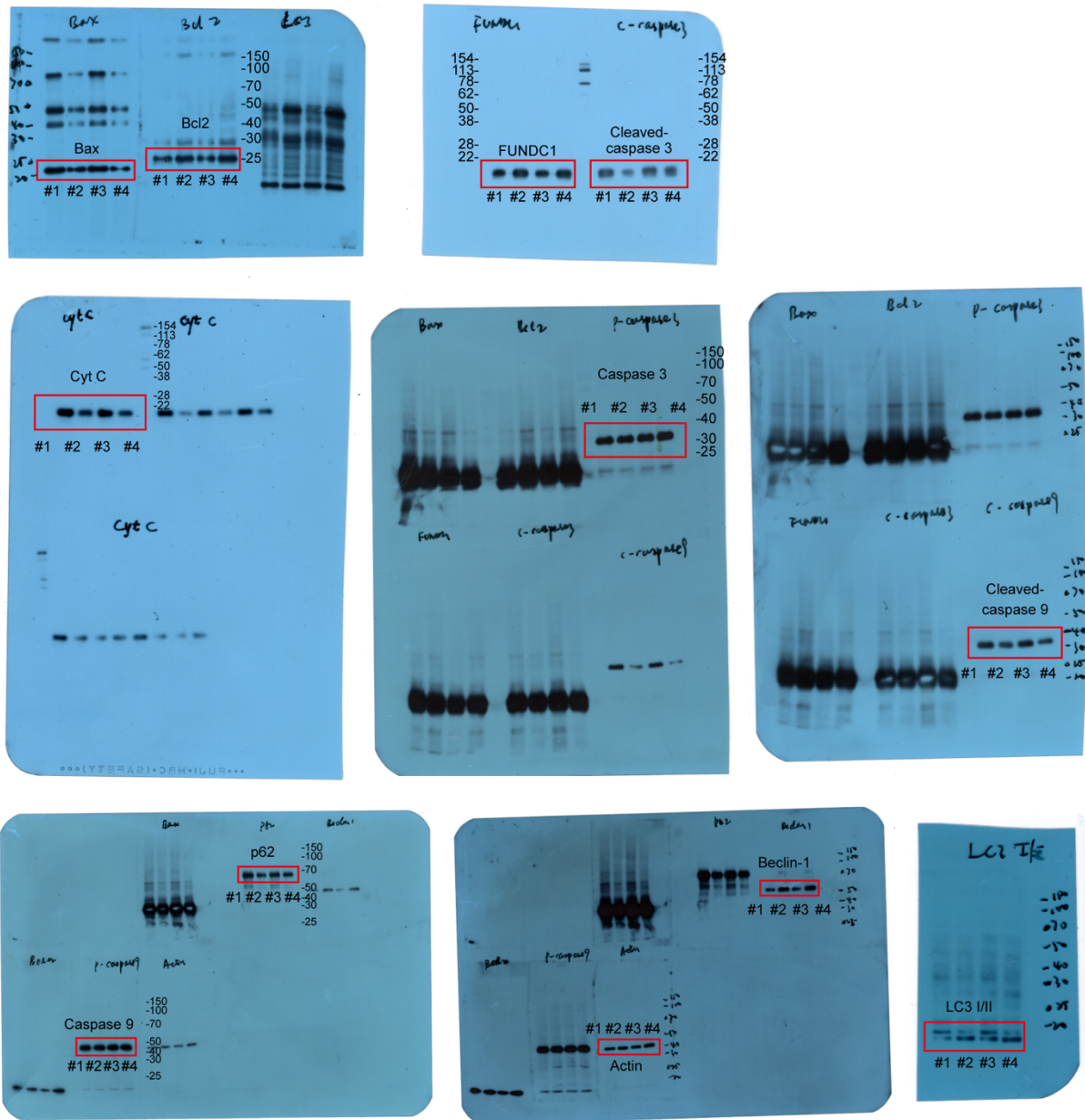


Fig S6. Uncropped blot results for Fig. 3A

#1 shNC
#2 shUSP35-1
#3 shUSP35-2

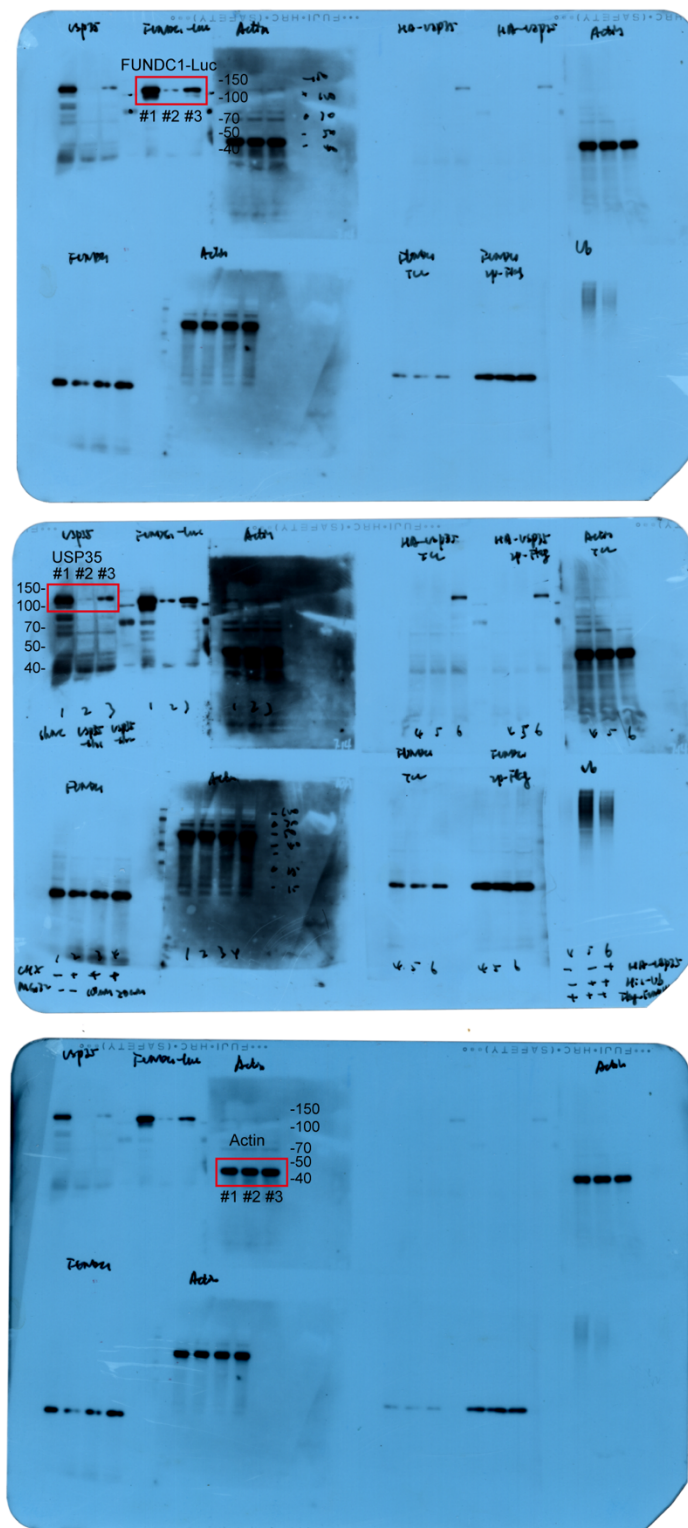


Fig S7. Uncropped blot results for Fig. 4B

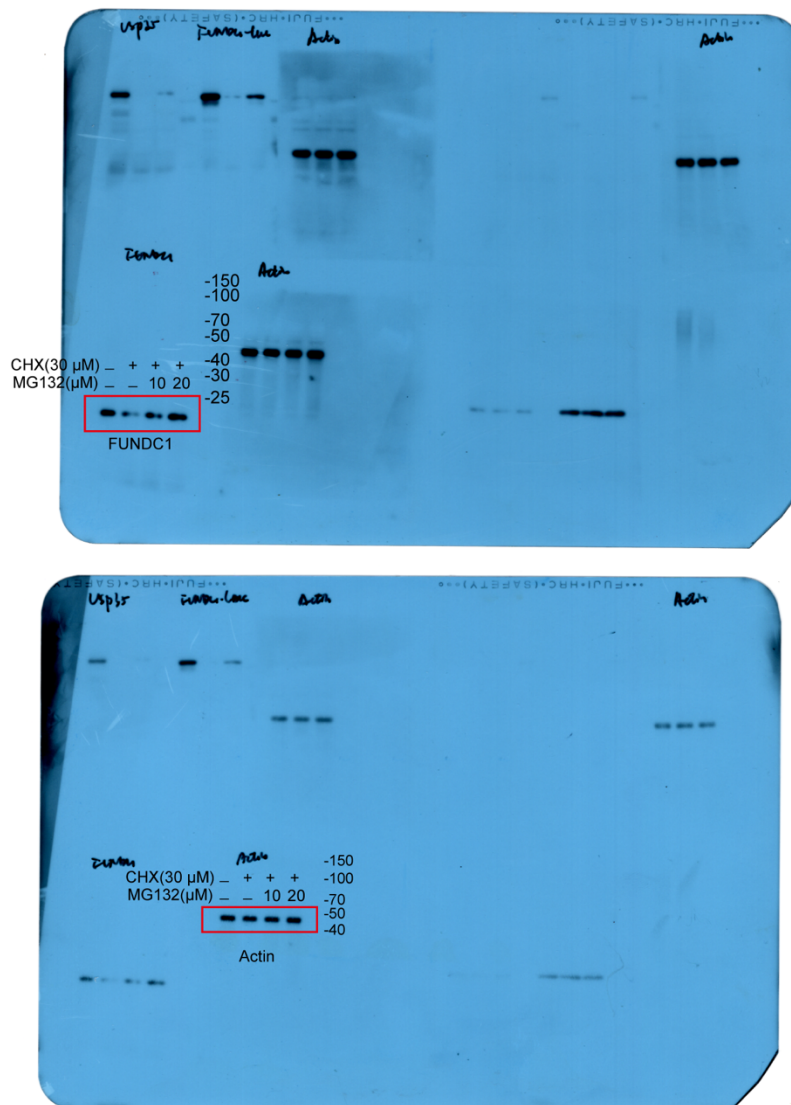


Fig S8. Uncropped blot results for Fig. 4C

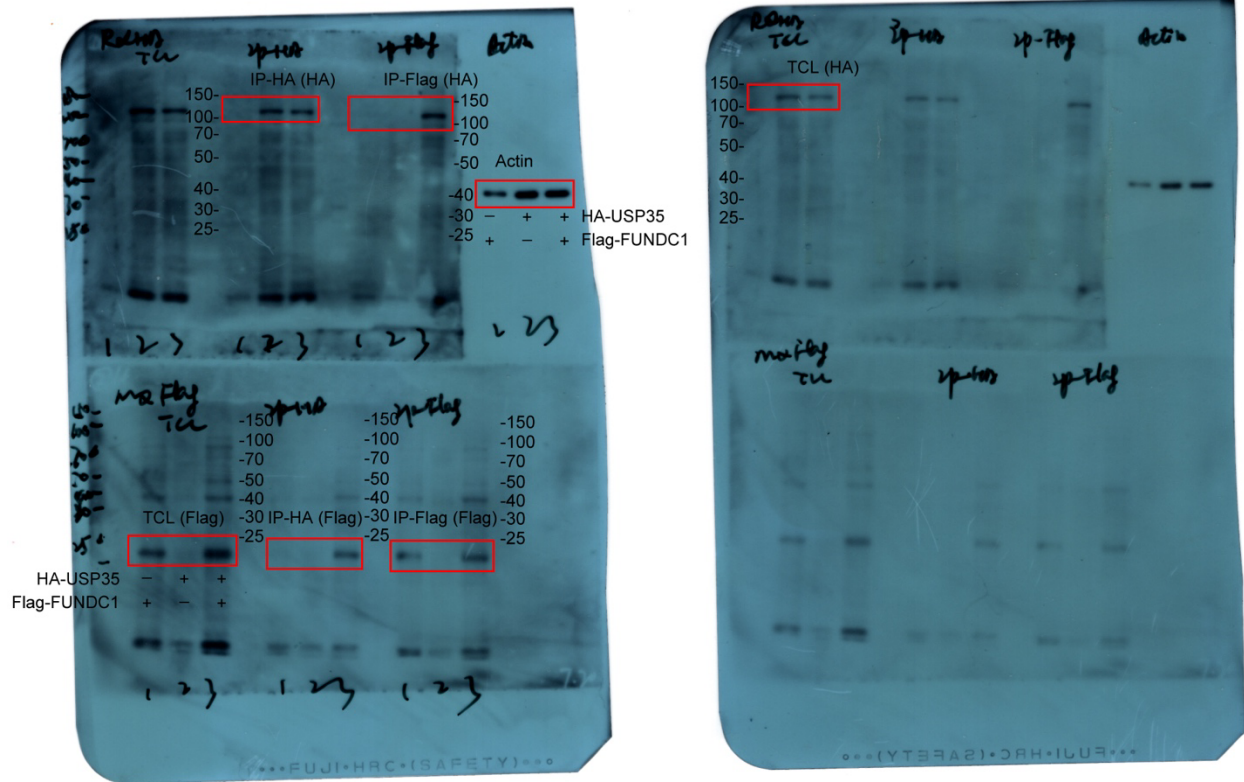


Fig S9. Uncropped blot results for Fig. 4D

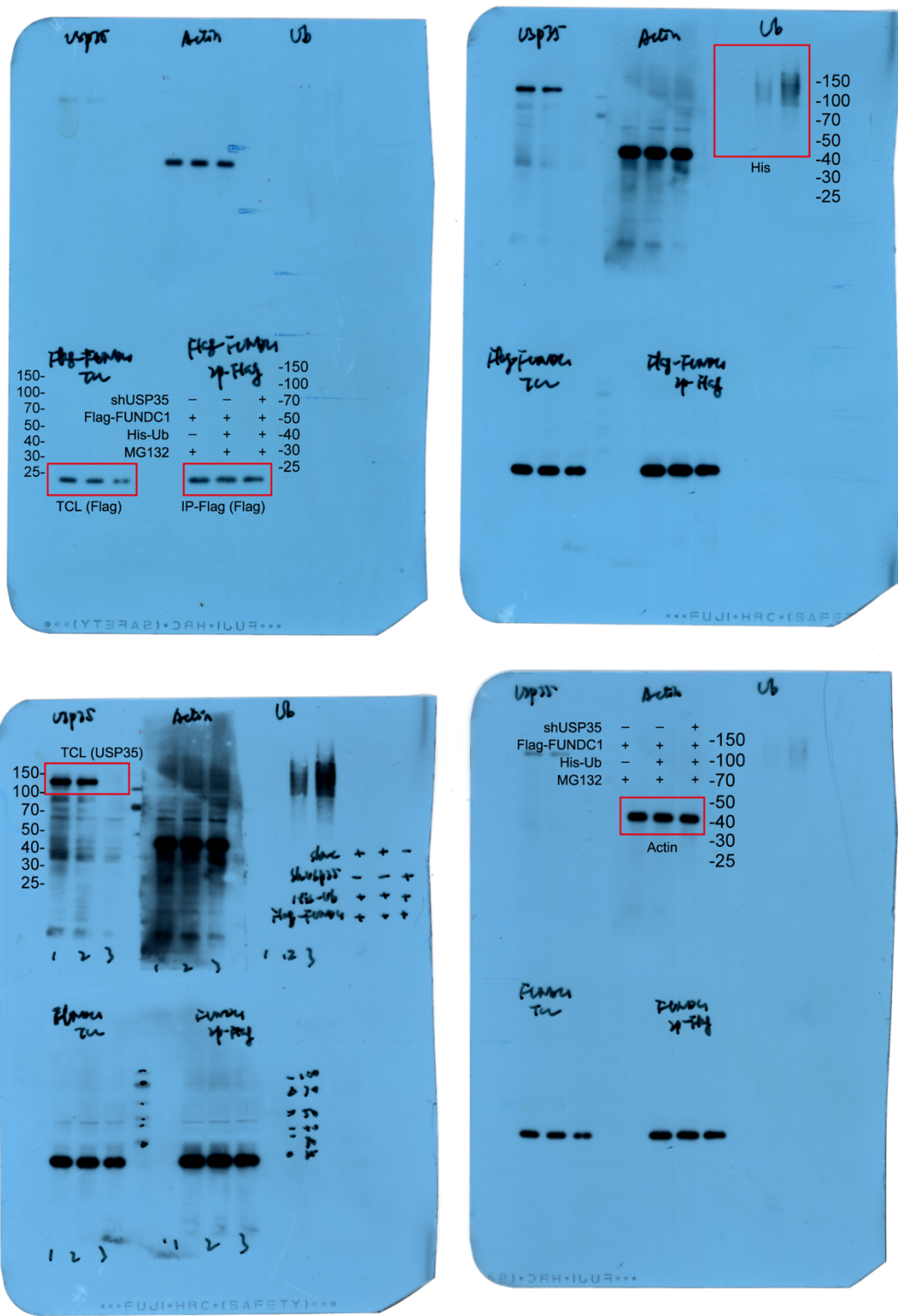


Fig S10. Uncropped blot results for Fig. 4E

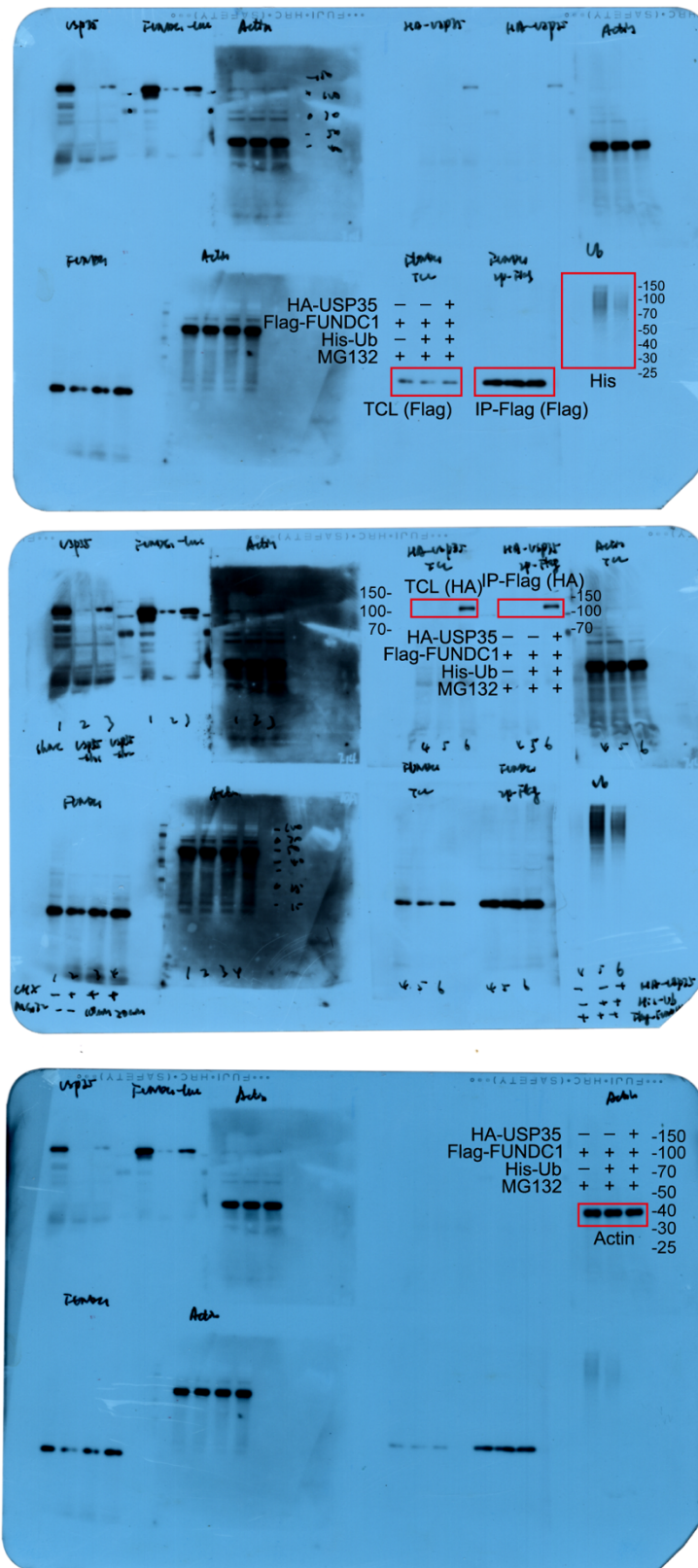


Fig S11. Uncropped blot results for Fig. 4F

shUSP35	-	-	-	-	+	+	+	+	HA-USP35	-	-	-	-	+	+	+	+
Flag-FUNDC1	+	+	+	+	+	+	+	+	Flag-FUNDC1	+	+	+	+	+	+	+	+
	#1	#2	#3	#4	#5	#6	#7	#8		*1	*2	*3	*4	*5	*6	*7	*8

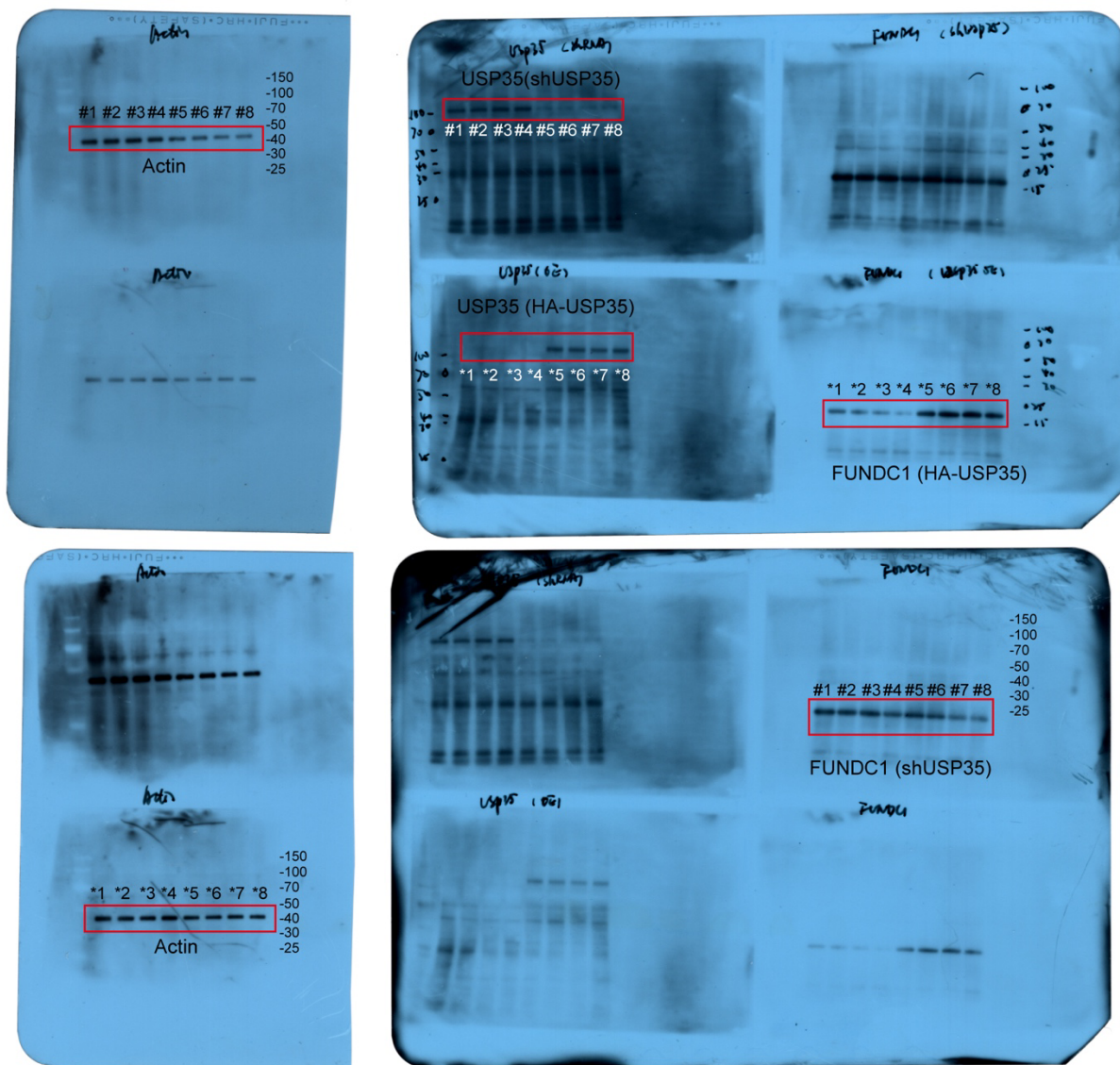


Fig S12. Uncropped blot results for Fig. 4G

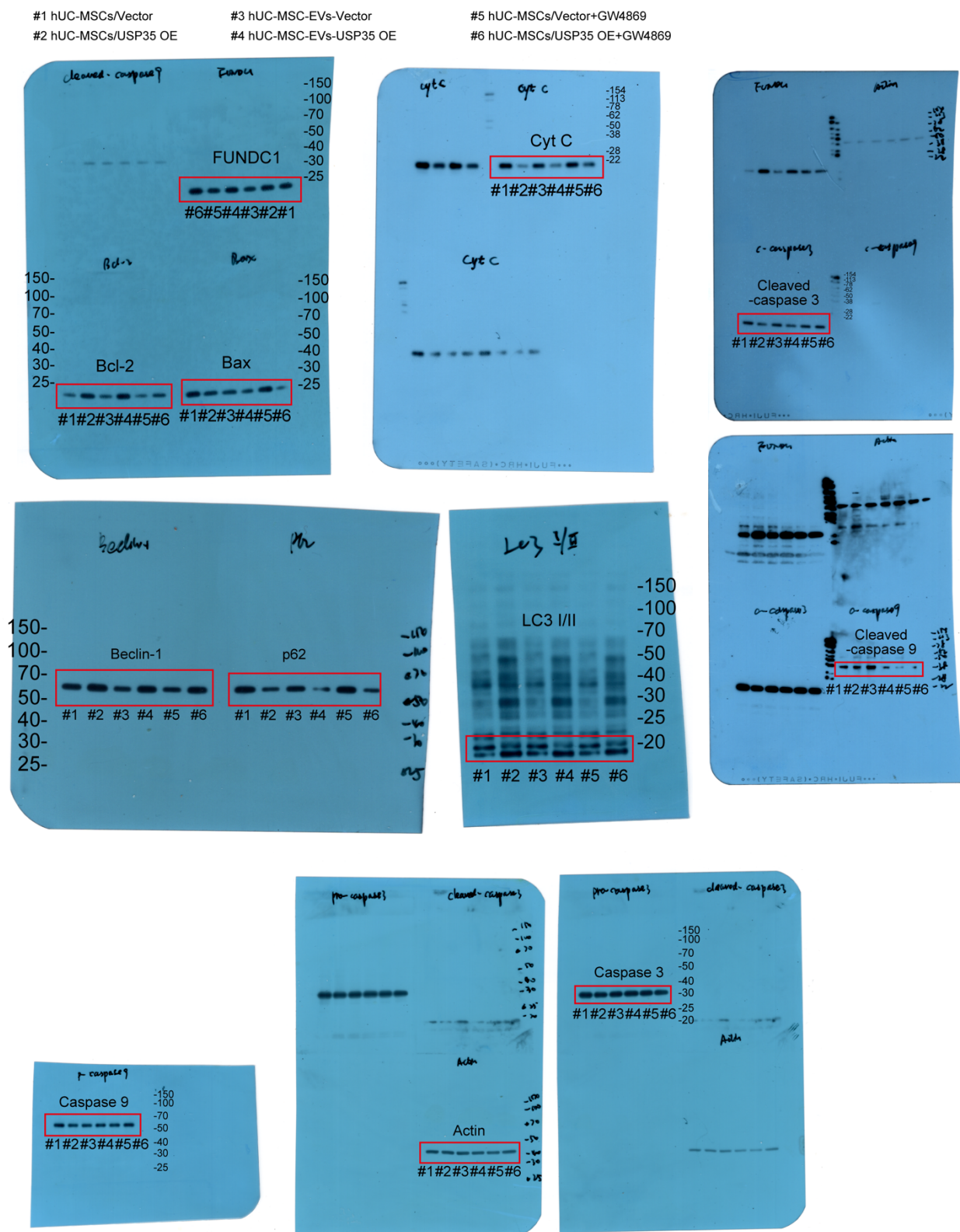


Fig S13. Uncropped blot results for Fig. 6A

#1 hUC-MSCs/Vector #2 hUC-MSCs/USP35 OE #3 shNC+hUC-MSCs/USP35 OE #4 shFUNDCl+hUC-MSCs/USP35 OE
 #5 hUC-MSC-EVs-Vector #6 hUC-MSC-EVs-USP35 OE #7 shNC+hUC-MSC-EVs-USP35 OE #8 shFUNDCl+hUC-MSC-EVs-USP35 OE

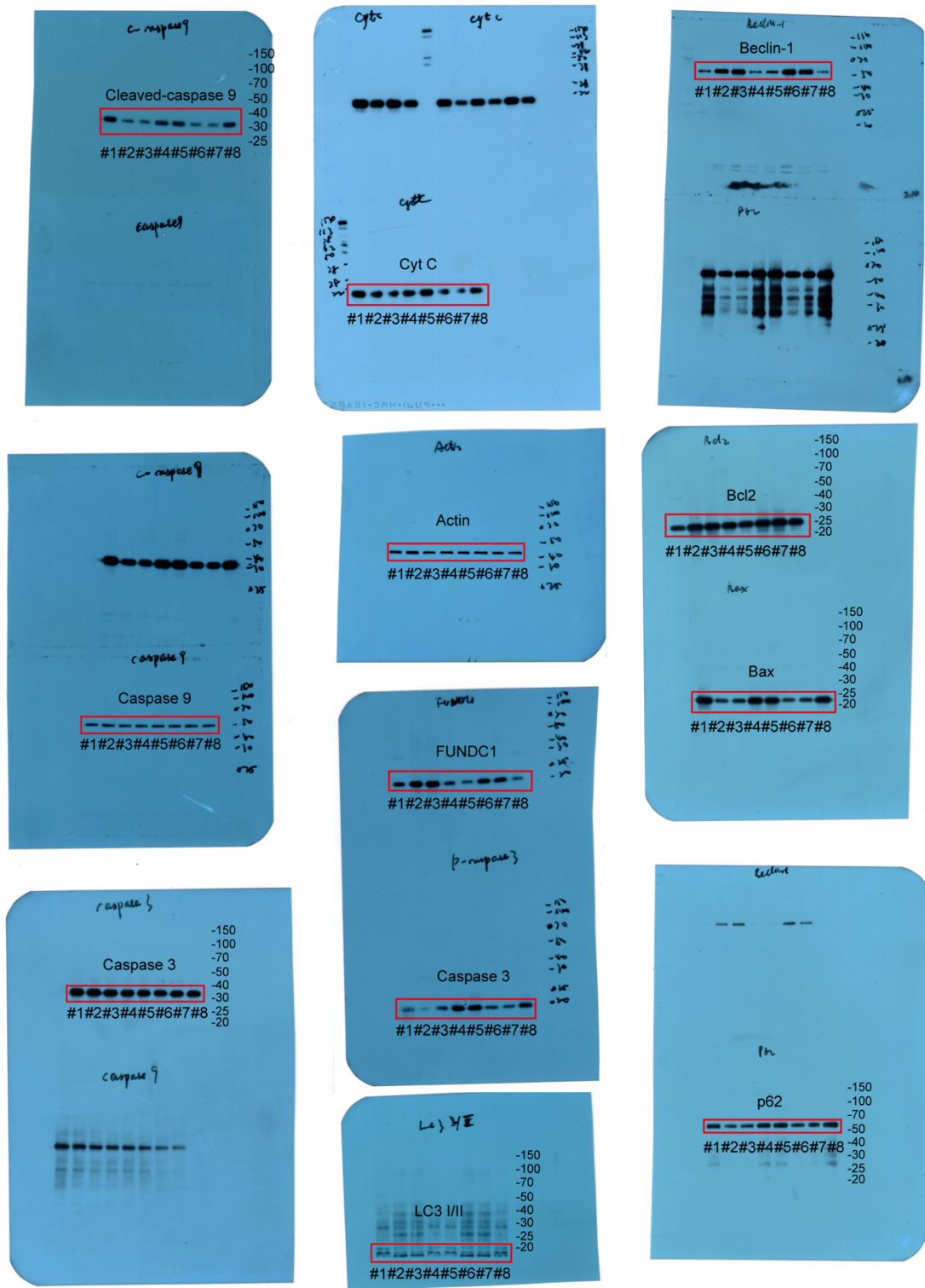


Fig S14. Uncropped blot results for Fig. 8A

#1 hUC-MSCs/Vector #2 hUC-MSCs/USP35 OE
 #3 hUC-MSC-EVs/Vector #4 hUC-MSC-EVs/USP35 OE

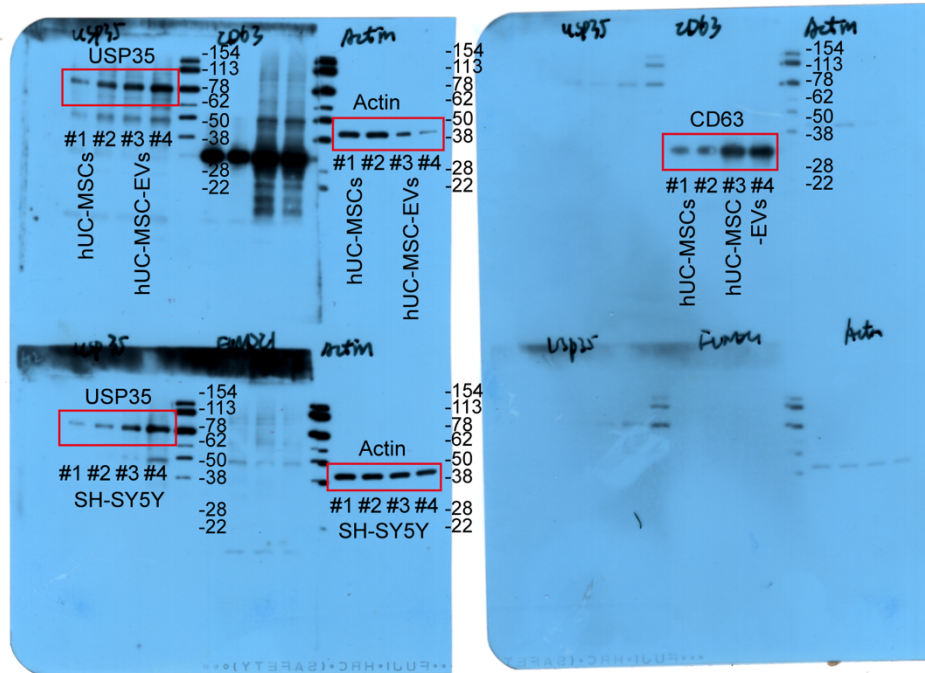


Fig S15. Uncropped blot results for Fig. S3B and C

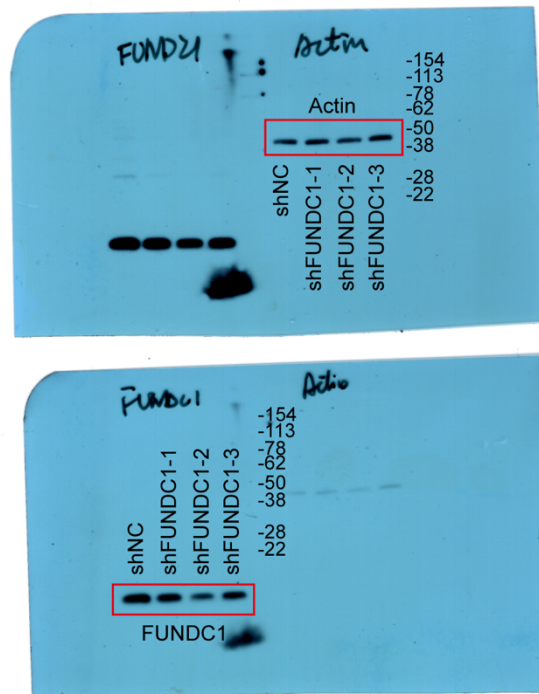


Fig S16. Uncropped blot results for Fig. S3E

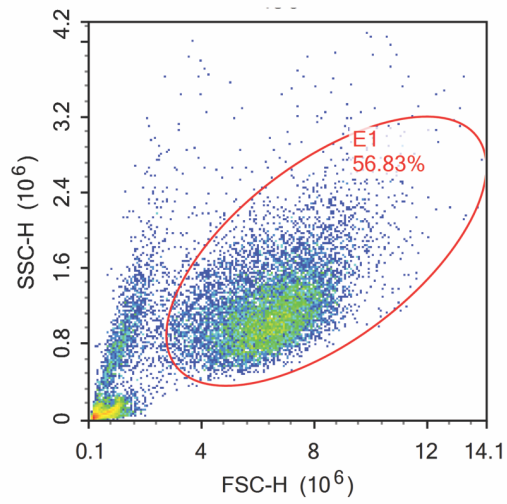
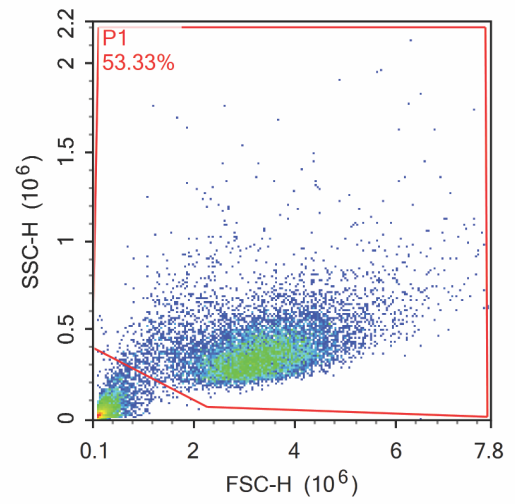
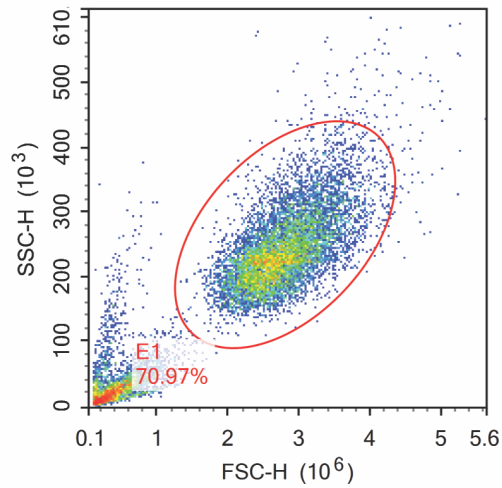
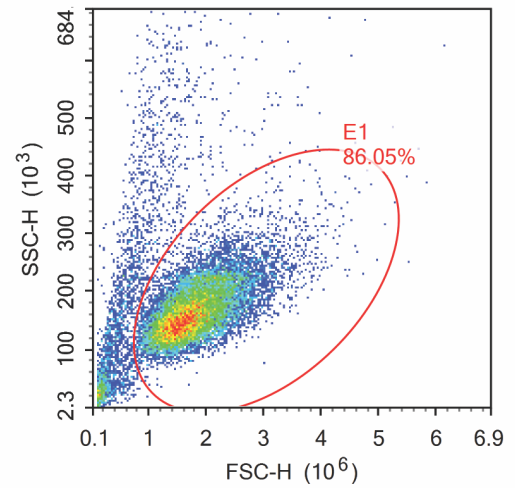
A**B****C****D**

Fig S17. Gating strategy for FACS. (A-D) Representative images showing the gating strategy for the identification of MSC surface marker (A), Annexin V/PI staining (B), JC-1 staining (C), and ROS level detection (D).